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THE ORIGIN OF THE EOSINOPHIL LEUCOCYTES OF MAMMALS

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The origin of the eosinophil leucocytes of mammals.

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With one plate No. I.

I. Introduction.

The origin of the eosinophil leucocytes of mammals has been a subject of controversy. At the present day there is no unanimity of opinion as to the true source and nature of the granules of these cells. There is, however, a tendency on the part of a number of haematologists to regard the eosinophil granules as composed largely of exogenous material. Weidenreich is the chief exponent of this theory; he maintains that eosinophil leucocyte granules are composed of haemoglobin or its dissociation products, which is taken up by non-granular cells and deposited later in the form of eosinophil granules. Weidenreich claims that this is their only source, and furthermore, he maintains that there are no observations on record which prove that eosinophil granules are differentiated gradually in the protoplasm of non-granular cells.

The following contribution represents a résumé of several years work in which an attempt has been made to analyze the origin of the eosinophil granules, and to determine to what extent they are related to haemoglobin or its products, under normal as well as under experimental conditions. For enthusiastic encouragement and most helpful criticism I wish to acknowledge my deep sense of appreciation to Professor Hal Downey, at whose suggestion this work was undertaken and under whose direction it has been carried on.

A survey of the voluminous literature on eosinophils shows that to H. F. Müller (1892) belongs the credit of describing for the first time

mitotic figures in the eosinophil leucocytes of mammals. Müller, however, did not maintain that regeneration of eosinophils in adult animals is necessarily limited to homoplastic mitosis of pre-existing eosinophil leucocytes. He simply showed that a homoplastic form of regeneration takes place. A number of other investigators, however, have emphasized homoplastic regeneration as the only form of development for the eosinophils of adult mammals.

van der Stricht (1892) also observed mitosis of eosinophil leucocytes, and stated that they are never derived from cells with homogenous protoplasm. He claimed that all of the eosinophils are derived from a granular parent-cell. Pappenheim (1899) also claimed that in the adult rabbit eosinophils are always regenerated from pre-existing eosinophils, although he admitted that in the embryo of the same animal a heteroplastic development might take place from non-granular cells.

Maximow (1907), although he noted heteroplastic development of granulocytes, stated that their regeneration in the normal adult is exclusively by homoplastic means. In subsequent investigations, however, he has shown that eosinophil myelocytes and mast myelocytes in normal adult bone marrow may also differentiate from non-granular cells.

Homoplastic regeneration in the marrow of adult animals is no longer doubted, but it is no longer believed, at least not by many, that it is the exclusive method for maintaining the requisite number of eosinophils. A number of investigators recognize that a heteroplastic means of regeneration must also be taken into account. Those who believe in heteroplastic development of eosinophil leucocytes, however, do not agree on the derivation of the granules which these cells contain, consequently a number of views have been advanced to account for their origin.

II. Literature.

According to Thayer (1897) and Brown (1898) there is direct transformation of neutrophil into eosinophil granules in the leucocytes of human muscle infected with trichinae. Klein (1899) believes that neutrophil leucocytes may take up haemoglobin from extravasated red cells and in so doing the neutrophil granules become changed into eosinophil granules. Mosny and Portocalis (1913) have recently again emphasized the view that eosinophil leucocytes are formed from neutrophils.

Many other investigators of the genesis of the eosinophil granules claim that they are of exogenous origin. In this connection the theories

of Tettenhamar (1893) and Sacharoff (1895) might be mentioned. The former claims that they consist of phagocytosed eosinophil substance which is thrown out from the degenerating nuclei of different types of cells. According to the latter author, eosinophil granules represent the phagocytosed nucleoli of haematoblasts. These views have never been accepted. The theory, nevertheless, that eosinophil granules represent exogenous material, has gained many ardent supporters.

Weidenreich's account of the origin of the eosinophil leucocytes as outlined in his book, Die Leukozyten und verwandte Zellformen, 1911, represents the consensus of opinion held by the majority of American and European haematologists, — namely that eosinophil leucocyte granules are composed of exogenous material which is related to haemoglobin or its dissociation products. This theory was advanced by Saltykow (1900), Stschastnyi (1905), Weidenreich (1901, 1905, 1908, 1910, 1911), Gütig (1907), Badertscher (1913/1915), and others, but, as previously stated, it has been most strongly supported by Weidenreich. His first observations were based on investigations of rat and sheep haemolymph nodes. In these mammals his findings favor the view that eosinophil leucocyte granules are haemoglobin-containing products of degenerating erythrocytes. According to Weidenreich, many of the free erythrocytes in the haemolymph nodes were found fragmenting into several small granules. He claims that these granules or fragments are later phagocytosed by non-granular cells whose nuclei change from the mononuclear to the bilobed condition. He maintains that the phagocytosed erythrocyte granules remain in the cell-body as true eosinophil granules, and that the change in the configuration of the nuclei of the lymphoid cells results in the formation of nuclei that are morphologically identical in appearance with the nuclei of the eosinophils of the blood. In this connection Weidenreich (1901, p. 197) states:

„Die eosinophilen Leukozyten sind also nichts anderes als sogen. Lymphozyten, welche die durch den Zerfall roter Blutkörperchen entstehenden feinen Trümmer in ihren Plasmaleib aufnehmen, wobei ihr Kern in die polymorphe Form übergeht."

He expresses a similar idea in his book (1911). On page 92 he states:

„. . . . . Die freien Granula nun umgeben die in den Bluträumen vorhandenen und in den anstoßenden lymphoiden Gewebe entstandenen granulafreien Leukozyten und werden von diesen aufgenommen; dabei erfährt der Kern eine charakteristische Umformung, indem er aus dem kompakten kugeligen Zustand zuerst in den bohnen-, dann in den huf-

eisenförmigen übergeht und schließlich gelappt wird, wodurch das bekannte Bild der eosinophilen Leukozyten entsteht."

Weidenreich claims that the haemoglobinous origin of the eosinophil leucocyte granules has been conclusively proved, and in defense of this theory he demonstrated his preparations at the sixteenth session of the Anatomischen Gesellschaft which met at Halle in 1902. In several subsequent publications (1905, 1910, and 1911) he refers to his findings in haemolymph nodes.

Ascoli (1904), however, examining similar material came to very different conclusions. He observed fragmentation of erythrocytes, and the phagocytosis of similar fragments, but he does not believe that phagocytosed erythrocyte fragments represent eosinophil leucocyte granules. Weidenreich (1905), on the other hand, believes that the exogenous origin of these granules has been proved by the observations of Lewis (1904), Sabin (1905), and Gütig (1907). On page 92 of his book he quotes Lewis in regard to the development of eosinophil leucocytes from lymphocytes that have phagocytosed erythrocyte fragments. So far as I am aware, however, and as previously pointed out by Schwarz (1914), Lewis does not claim that eosinophil leucocyte granules are formed from erythrocyte fragments. As far as the real origin of these granules is concerned there is frank admission on the part of Lewis that there are several arguments, as well as good evidence, which still leave the genesis of the eosinophil granules an open question.

Sabin (1905) does not attempt to prove that eosinophil granules are formed from erythrocyte fragments, although she states that these fragments can not be distinguished from the granules of eosinophil leucocytes. Gütig (1907), however, described the development of eosinophil leucocyte granules from phagocytosed erythrocyte fragments in the mesenteric lymph nodes of adult pig. These leucocytes were smaller than those found in the circulating blood, and according to Gütig they never get into the blood-stream. He claims that there are two kinds of eosinophils, those of the tissues, and those of the blood. The granules of the former are the phagocytosed fragments of erythrocytes, the granules of the latter represent true cytoplasmic differentiations.

Badertscher's (1913/1915) observations have given additional support to Weidenreich's theory. His views are based on a study of amphibian material (Salamandra atra). The gill muscles and a number of other muscles were found to undergo degeneration during metamorphosis. He also observed degeneration of blood capillaries with pronounced

destruction of erythrocytes in the degenerating gills and muscles. Badertscher maintains that the degeneration of muscle tissue, and the destruction of erythrocytes result in the formation of free granules. Like ordinary eosinophil leucocyte granules, they stain in eosin. These free granules are phagocytosed by „cells of the character of leucocytes" and thereby changed into eosinophil granules. On this basis Badertscher maintains that he can account for the numerous eosinophil leucocytes in certain degenerating tissues, aud in the blood during the period of metamorphosis.

In connection with an investigation on the development of the thymus in the pig, Badertscher (1915) reiterates the view that the granules of eosinophil leucocytes are of exogenous origin and that they are related to haemoglobin. He noted considerable degeneration of erythrocytes in the mesenchyme of early stages and also in the interlobular septa of later developmental stages. The lobules of the thymus also showed degenerative changes on the part of the free erythrocytes. As a result of degeneration groups of free eosinophil granules were observed in the thymus wherever there was development of red cells. Badertscher admits that free eosinophil granules were never numerous, although in late developmental stages of the thymus, which contained numerous erythrocytes, they were more abundant. Lymphocytes, according to the author, take up the free eosinophil granules and become transformed into eosinophil leucocytes.

Weidenreich's experimental work on the eosinophil problem is in all probability of greater importance than are his significant findings in haemolymph nodes. He, however, was not the first investigator to attempt the solution of this problem by the experimental method, for in the year 1905 Stschastnyi attacked the problem experimentally. He injected erythrocytes of the cat, dog, and rabbit into the peritoneal cavity of the guinea pig. A single intraperitoneal injection of erythrocytes produced no appreciable increase in the number of the guinea pig's eosinophil leucocytes. There was, however, a marked increase in the number of these cells after a third or fourth injection. Stschastnyi explains the increase in their numbers on the theory that foreign erythrocytes, when introduced into the peritoneal cavity, are phagocytosed by large mononuclear cells and broken up into eosinophil granules. He admits that fragmentation of erythrocytes does not result in the formation of granules which are identical in character with eosinophil leucocyte granules, and he also admits that he was unable to find a complete series of intermediate stages. Irrespective of his findings he believes, nevertheless, that eosinophil leucocyte granules are formed from

breaking down erythrocytes. In this connection Stschastnyi (p. 476) states:

„Es ist viel Grund für die Annahme vorhanden, daß das Anwachsen der Zahl der Eosinophilen in engen Zusammenhang mit der Aufnahme solcher Körnchen und dem Zerfall der Erythrozyten innerhalb der Leukozyten steht, doch muß ich zugeben, daß es mir auch nicht ein einziges Mal gelungen ist, auf dem heizbaren Objektionstisch im hängenden Tropfen den Übergang solcher von Leukozyten aufgenommener pseudoeosinophiler Körnchen in echte eosinophile zu sehen, oder eine Veränderung und wenn auch nur in der äußern Gestalt der Körnchen innerhalb der Leukozyten wahrzunehmen. Trotzdem glaube ich, daß es die Tatsachen — das dem Verschwinden der freien Körnchen proportionale Anwachsen der Anzahl der Eosinophilen im Exsudat und das Auftreten von Mono- und polynukleären Übergangsformen der Eosinophilen mit spärlichen Granulationen — gestatten, in den in Auflösung begriffenen Erythrozyten das Bildungsmaterial für die eosinophile Körnelung zu sehen."

From the contents of the above quotation, as well as from the body of the paper, it appears that Stschastnyi's view is open to real criticism. He evidently anticipated objection to his theory, for he states that haemoglobin granules are not identical with eosinophil leucocyte granules, and furthermore, that he did not observe the transformation of erythrocyte fragments into eosinophil granules; and so he constructs another theory. According to this theory, the leucocytes which contain erythrocyte fragments or granules break down. These free granules are then taken up by other cells and transformed into real eosinophil leucocyte granules. He admits, however, that he did not see the essential intermediate stages in such a transformation process.

Weidenreich's experiments consisted in the repeated injections of guinea pig erythrocytes, at definite intervals, into the peritoneal cavity of rabbits. The results which he obtained were similar to Stschastnyi's, although Weidenreich's observations appear more positive in character and have been generally accepted. His findings have been submitted as conclusive proof for the haemoglobinous origin of the eosinophil leucocyte granules. He bases his view on the findings in the omentum following the repeated intraperitoneal injections of red cells. Following these injections Weidenreich claims that the lymphocytes of the tâches laiteuses of the omentum become changed into eosinophil leucocytes. He states that from 40 to 50% of these cells were of the mononuclear type, and that he could trace all the intermediate stages

between these and the fully differentiated eosinophil leucocytes with polymorphic nuclei. The mononuclear eosinophils, according to Weidenreich, are rather localized in the omentum, and their nuclei have the characteristic earmarks of small lymphocyte nuclei. He interprets the mononuclear forms as the real source of the eosinophils of the omentum, and believes that they, together with the fully developed eosinophil leucocytes with bilobed nuclei, furnish conclusive evidence for an autochthonous origin of eosinophils. In support of such a local development Weidenreich (1908, pp. 84 and 85) cites the following five points:

1. das vollständige Fehlen jeder entzündlichen Reizung und das durchaus normale Verhalten der Blutgefäße des Netzes, die im vorliegenden Falle überhaupt keine eosinophile Leukozyten und nur die normale Anzahl feingekörnter (pseudoeosinophiler) enthielten; 2. die Anwesenheit vieler Tausende von eosinophilen Leukozyten in Netzpartien, in denen Blutgefäße, auch Kapillaren, absolut fehlen; 3. die Tatsache, daß die Hauptmasse der eosinophilen Elemente kompaktkernigen, d. h. nach alter Benennung mononucleären Charakter aufweisen, während gerade solche Formen in der Zirkulation zu den Seltenheiten gehören; 4. das örtliche Zusammenfallen der eosinophilen Zellhaufen mit den Tâches laiteuses und die direkt zu konstatierende Substitution der Lymphozyten dieser Milchflecke durch die granulierten Elemente; 5. endlich die Möglichkeit, die Umbildung der typischen Lymphozyten in ebenso typische eosinophile Leukozyten schrittweise zu verfolgen.

Sternberg (1914) repeated Weidenreich's experiments by injecting suspensions of sheep erythrocytes into the peritoneal cavity of guinea pigs. Following the injections there was a great increase in the number of eosinophils of the peritoneal cavity. Fragments of erythrocytes were observed in many of these cells. Sternberg maintains, however, that he can always distinguish erythrocyte fragments from eosinophil leucocyte granules. This distinction is possible in both fresh and stained preparations. He states that erythrocyte fragments are characterized by being larger than are the leucocyte granules. Furthermore, he observed no intermediate stages which would indicate that the former are converted into the latter type of granulation. The omentum showed nothing of special interest, and this was also true of the liver, kidney, and lung. The number of eosinophils in these structures was never of great consequence; myelocytes were not observed. The spleen, however, contained numerous eosinophils, and the marrow was very active in the production of these cells; here they were more numerous than the special cells. Sternberg, concludes, therefore, that the eosinophil leucocytes are specific cells of the marrow. He claims that

the increase in the number of eosinophils of the peritoneal cavity, following the repeated injections of foreign erythrocytes, must be interpreted as a response to anaphylactic conditions and not to autochthonous differentiation.

Weidenreich maintains that in all mammals the eosinophil leucocyte granules are of haemoglobinous origin. He assumes that even in the marrow the granules of the corresponding myelocytes are haemoglobin-containing products of degenerated erythrocytes. This assumption is based on the well established fact that degeneration of erythrocytes is a normal process in the marrow as well as in other haematopoietic organs. Weidenreich assumes that in instances where fragmentation of these cells is not apparent that their haemoglobin passes out of the cell-body in solution. The free haemoglobin, according to his theory, is subsequently taken up by lymphoid cells in the marrow, worked over and deposited in the form of eosinophil granules.

Observation of the phagocytosis of free haemoglobin is not wanting. Brass (1913) reports the presence of pigment in the endothelial cells of the capillaries in rabbit bone marrow. The endothelial cells take up the free haemoglobin and deposit it in the form of pigment granules. In this connection the findings of Marwedel (1897) are of interest, especially because he finds that eosinophil leucocytes may contain pigment granules which are identical in character to those frequently resulting from the breaking down of haemoglobin. Intermediate stages were observed between eosinophil and pigment granules which indicate that the latter were derived from the former. The pigment granules responded to the iron test. Marwedel believes that eosinophil leucocyte granules are related to haemoglobin, although not derived from it. According to him, the eosinophil granules represent true cytoplasmic differentiations.

Arnold (1913) also believes that eosinophil leucocyte granules are true endogenous differentiations of the cells that contain them. Their constitutional makeup, however, may vary from time to time, since they are able to take up exogenous material such as haemoglobin, iron, etc. The above mentioned pigment granules in the eosinophils of rabbit marrow (described by Marwedel) are explained on the assumption that the "Plasmosomes" of the eosinophils have taken up haemoglobin in solution and changed it into haemosiderin.

Further observations on the formation of pigment from haemoglobin in solution are found in numerous investigations. Chevallier (1914) reports that pigment cells are formed in the spleen by cells that have taken up iron. From the spleen the pigment cells may migrate

into other parts of the body. Chevallier believes that the pigment granules represent iron which has been derived from haemoglobin. In case the spleen is removed the iron is taken care of by the intestinal villi, omentum, and mesenteric and aortic lymph nodes. Kyes (1915) found that phagocytosis of erythrocytes, with subsequent formation of haemosiderin from the haemoglobin, is a normal process for the stellate cells of the liver and the reticular cells of the spleen pulp of frog, Bufo, turtle, crocodile, pigeon, and oppossum. Hooper and Whipple (1916) showed that haemoglobin solutions when introduced into the circulation, the liver being cut off, and also when introduced into the peritoneal and pleural cavities, may be converted into bile pigment. In the peritoneal cavity most of the haemoglobin solution is absorbed very rapidly; so the bile pigment appears in the urine. In the pleural cavity, on the other hand, where there is relatively a small amount of absorption, there is a relatively small amount of haemoglobin appearing in the urine following intrapleural injections of haemoglobin, due to the fact that absorption from the pleural cavity is slow.

The above mentioned authors found no relationship between haemoglobin or the pigment derived from it and eosinophil leucocytes.

Arnold (1895), in studying rabbit marrow, observed basophilic granules in many of the granulocytes, some of which contained both basophilic and eosinophilic granules in their cytoplasm. He assumed that the basophilic granules were transformed into acidophilic granules, because great numbers of the former showed considerable variation in their staining reactions. Arnold, however, did not attempt to determine the significance of these variations for a better understanding of the acidophil granules.

Benacchio (1911), Kardos (1911), and Pappenheim-Szécsi (1912) did not make a special study of the histogenesis of the eosinophil leucocytes, but in connection with their studies on the origin of mast leucocytes in rabbit marrow, Benacchio and Pappenheim-Szécsi stated that all of the myelocytes which contain basophilic granules represent the "unripe" stages of young eosinophils and pseudoeosinophils. Benacchio failed to find myelocytes in which all the granules were basophilic in either rabbit or guinea pig bone marrow. In connection with my findings in rabbit marrow, which in general have been made known through previous preliminary notes (1915a/1915b), I considered the inadequacy of Benacchio's technique for the finer histological study of the granulocytes, with particular reference to the mast myelocytes. In this same connection the work of Pappenheim and his students, Kardos and Szécsi, was subjected to analysis.

Maximow, in his earlier studies of the marrow, was interested mainly in the rôle which lymphocytes play in the elaboration of granules in their cytoplasm, and consequently, he did not attempt to make a detailed investigation of the genesis of the eosinophil series. In 1913, however, his figures show that the very youngest granules of eosinophil myelocytes are basophilic when first differentiated in the cytoplasm, and that they are gradually transformed into acidophil granules, although Maximow does not emphasize this point.

Downey (1914/1915) made a special study of the life-history of the eosinophil granules, in the bone marrow of adult guinea pig, and noted variations in the staining reactions and morphology of these granules. He also reports that eosinophil granules are basophilic when first formed. They gradually change their staining reactions, taking on the eosin of the indulin-aurantia-eosin mixture. In the later stages of differentiation the granules lose their avidity for the eosin of the staining mixture and stain with the aurantia of the same combination. With reference to these staining reactions Downey (1914, p. 136) states:

". . . . . Some of these granules change their staining reactions while they are still small and basophilic, while others remain basophilic until they have reached a size even greater than that of the fully differentiated granule before such change takes place. That these larger granules do not disappear, and that they are transformed directly into the eosinophil granules is shown by the fact that many of the largest ones are stained in the acid component of the staining mixture, while others are of a mixed tone."

Downey maintains that these gradual progressive changes in the staining reactions of the basophilic granules, in the eosinophil myelocytes, together with changes in their configuration, prove that eosinophil granules are endogenous differentiations. "Their complex evolution indicates that they are true endogenous formations" (1915 p. 199). Downey's results show still further, in contradistinction to the claims of Browning (1905), Sternberg (1905), Helly (1906/1910), and Grawitz (1911), that there is also heteroplastic differentiation of eosinophils from non-granular cells.

Outside the marrow it is reported by Dekhuyzen (1891) that under normal conditions eosinophil leucocytes may be regenerated by homoplastic means. Numerous other investigators, however, have proved that heteroplastic differentiation must also be taken into account. Unfortunately, however, most of these observations are concerned with the general features of the heteroplastic differentiation of the granular leucocytes, and little is said about the origin and character of their

granules. In instances where the granules have received attention, mention will be made of it in discussing the literature.

Ehrlich (1878/1879) reported extramedullary differentiation of eosinophils in the connective tissues of the frog, particularly in its mesentery. He claimed, however, that these leucocytes were of very different character from those present in the blood-stream.

Heteroplastic development of eosinophils in the tissues has also been described by Pappenheim (1905), and Dominici (1909). The latter finds, after intraperitoneal injections of taenia extract into adult rabbits, that many lymphocytes of the omentum are transformed into eosinophil leucocytes. Dominici believes that the local eosinophilia in this case is due to the chronic irritation of the extract on the omentum. Howard and Perkins (1902) find, in chronic inflammatory conditions, that eosinophil leucocytes may arise in loco from large mononuclears and plasma cells. Weill (1913) believes that the granular leucocytes in the thymus of homo and rat arise in loco from lymphocytes that have differentiated granules in their protoplasm. Although he does not emphasize the endogenous origin of the eosinophil granules it appears that they are real cytoplasmic differentiations.

Weidenreich and Badertscher, as previously mentioned, emphasize the local development of eosinophils from non-granular cells which have taken up haemoglobin.

Barbano (1914) believes, that in local eosinophilia, he can exclude the emigration of myelocytes from the vessels, and that the cells which are found in local accumulations are, therefore, new differentiations from non-granular cells. The latter are typical small and large lymphocytes. That they may differentiate into granulocytes is shown by the presence of many mononuclear eosinophils whose nuclei are identical with those of the lymphocytes. Barbano, as far as I am able to ascertain from a study of the literature, is the only author who has noted differences in the staining reactions of eosinophil granules in cases of local eosinophilia. He does not, however, give detailed descriptions of these changes. Neither does he interpret these differences as indicative of a gradual "ripening" process on the part of the eosinophil granules. His study of the subject indicates merely that the granules of the tissue eosinophils are quite variable in their staining capacity with eosin. These variations, however, do not appear to be associated with any particular stage in the development of eosinophils. There is no indication to warrant the belief that those granules which show the least affinity for eosin are the youngest ones.

Hartmann (1915) maintains, in contradiction to Weidenreich,

Badertscher, and Weill, that the eosinophils which are present in the developing thymus of rabbit are not autochthonous elements. Although she admits the presence of numerous mononuclear eosinophil myelocytes, she claims that there was no indication that their granules were gradually differentiated in the protoplasm of non-granular cells. The cells were filled with granules or they contained none. There were no cells containing only a few granules. According to Hartmann, the eosinophil myelocytes do not originate in the thymus.

The theory of the local development of eosinophils has found additional support in the studies of Herzog (1915). He claims that eosinophil myelocytes are present normally in small numbers in the mesentery of the guinea pig. Under inflammatory conditions, produced experimentally, Herzog found that the myelocytes were very numerous in the tissue immediately surrounding the foreign body, and also in the neighboring mesentery and omentum. Both eosinophil and pseudo-eosinophil myelocytes were present; it was often very difficult to distinguish the one from the other. The granules of the mature eosinophil leucocytes are rod-like in form, while those of the immature cells are plumper and shorter and may be grouped in pairs. Herzog claims that both large and small eosinophil myelocytes may originate from adventitial cells. Large wandering cells may also differentiate eosinophil granules. He believes that the large eosinophil myelocytes grouped about small vessels in the cortex of lymph nodes are related to the endothelial cells of the vessels.

Local development of eosinophils, however, is still denied by a number of authors among whom may be mentioned Helly, Schridde, Walter Fischer, and Maximow. Maximow's (1905, p. 753) conception of the tissue eosinophils is as follows:

„Die im Bindegewebe vorkommenden eosinophilen Zellen sind gewöhnliche aus den Blutgefäßen emigrierte eosinophile Leukozyten. Für eine lokale Entstehung im Gewebe fehlt jeder Beweis. Sie bleiben im Bindegewebe vermutlich lange Zeit liegen, als vorübergehend fast oder ganz unbeweglich gewordene Zellen; ein Teil kann sich später vielleicht wieder in die Blutbahn entfernen, ein anderer verfällt der Degeneration."

Maximow denies emphatically any genetic relationship between the eosinophils and the other cellular elements of the loose connective tissues. In this connection he states (1905, p. 748):

„Es ist nicht die geringste Spur einer genetischen Beziehung der eosinophilen Zellen zu den anderen Zellformen des Bindegewebes vor-

handen . . . . . Daß die Emigrationsbilder nicht zu finden sind, wird natürlich auch niemand ernstlich als einen Gegenbeweis ansehen."

The present study on the origin of eosinophil leucocytes in mammals is concerned with a number of more or less distinct fields of investigation. In any attempt to elucidate the life-history of these cells, the bone marrow should obviously receive first consideration. If the eosinophil granules are derived from haemoglobin products, as maintained by Weidenreich and others, there should be substantial evidence of the process in the marrow, where the predisposing conditions for the differentiation of eosinophil leucocytes, and their precursors, the eosinophil myelocytes, must be constantly at work.

Before discussing the eosinophils it is necessary to consider first the mast leucocytes and their supposed relations to eosinophils and other leucocytes. The necessity of such consideration is apparent when one recalls that Pappenheim and his students maintained that rabbit mast leucocytes are not true granulocytes, but lymphocytes whose spongioplasm has undergone a form of mucoid degeneration. The mast leucocytes of the guinea pig, on the other hand, are supposed to represent true granulocytes, but not true mast cells. The mast cells of this animal are regarded as the basophilic, "unripe" stages of eosinophil and special cells which have been thrown into the blood-stream before becoming completely differentiated. These cells have polymorphous nuclei, while the so-called true mast leucocytes of the rabbit have nuclei whose configuration corresponds to that of the lymphocyte nucleus. Pröscher had previously emphasized that the mast leucocytes of the rabbit were of the mononuclear type, and Pappenheim made a similar statement. Maximow, however, has shown that the mast leucocytes of the normal rabbit are always of the polymorphous type.

Maximow, Downey, and the author have proved that the mast leucocytes of mammals represent an independent line of granulocytes which is in no way related to the eosinophil or special leucocytes excepting through the non-granular parent-cell of the bone marrow. In the blood of the normal adult rabbit, mononuclear mast leucocytes are never found. These cells, therefore, do not show the relationships to lymphocytes of the circulation as described by Pröscher and others. In the rabbit mast granules are strongly basophilic from the moment of their first appearance in the cell-body, and they remain so throughout the life of the cell. They are of very different character from that of the basophilic granules of eosinophil and special myelocytes. For the guinea pig, Downey states:

". . . . . With this or any other staining mixture (abklatsch preparations fixed in Helly's fluid) it is evident that the shape of the mast granules is very different from that of the basophilic granules of the eosinophil myelocytes. A comparison of the granules of any one of the mast cells shown in figs. 11 to 16 and 18 to 24 with the eosinophil myelocytes of figs. 6 to 9 is sufficient proof of this. The great majority of them are elongated oval or pointed structures, while the unripe eosinophil granules are always round. The variations in size of the latter are also very much greater. Small granules are also found in some of the mast cells, but the majority of the granules in any one cell are about equal in size. There is absolute uniformity in the staining reactions of the mast granules when the triglycerine or triacid mixtures are used, while the unripe eosinophil granules show great variation in this respect."

Maximow also states that the basophilic granules of eosinophil myelocytes are of a different type, and can always be distinguished from mast cell granules. Poor technique seems to be responsible for the conclusions of some writers. Since both Downey (1915, pp. 175 to 176) and Maximow have discussed the inadequacy of the methods embloyed by Benacchio and Kardos, it is unnecessary to consider this question here.

Weidenreich admits that eosinophil myelocytes may contain basophilic granules, but he regards them as representing either endogenous granules which are not related to eosinophil granules or as the remains of nuclei that have undergone degeneration. He does not believe that eosinophil granules are basophilic when they are first differentiated in the cytoplasm of the myelocyte.[1])

Grawitz (1911) also maintained that there is no relationship between basophilic granules and those that stain with eosin in eosinophil myelocytes. He stated emphatically that the gradual differentiation of eosinophil granules in the protoplasm of lymphoid cells had never been observed.

According to Browning (1905), there is some uncertainty as to the early basophilic condition of young eosinophil granules. He is inclined to believe that if this were true basophilic granules would be more numerous in young eosinophil myelocytes.

Thus it would seem that the basophilic condition of young eosinophil

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[1]) Weidenreich believes that if all of the granules of the eosinophil and special leucocytes are basophilic when first differentiated, it would be impossible to distinguish their myelocytes from the basophilic myelocytes of mast leucocytes.

granules is open to question, although it is generally admitted that eosinophil myelocytes may contain basophilic granules.[1])

An early "primitive" granulation which is also basophilic has been reported by several authors. Pappenheim (1912 and earlier) regarded it as a "prodromale" granulation which was not related to the eosinophil or special granulation. According to Pappenheim, these prodromale granules were derived from the nucleus of the cell, and they too were basophilic when first differentiated, but not metachromatic. He believed that the prodromale granules had no relationships with the final eosinophil or special granules which developed later and replaced the prodromale granules. Maximow also believes in the presence of a primitive granulation in the special myelocytes of the rabbit. He regards it as an azurophil granulation, and claims that it is not related to the specific granulation which develops during the disappearance of the primitive granulation. Downey (1913) also describes a primitive type of granulation in clasmatocyte-like cells of the guinea pig that are developing into histogenous mast cells.

A number of authors believe that basophilic granules represent the youngest eosinophil granules, while others maintain that their basophilic staining reaction is evidence of regressive changes in these granules. According to Ehrlich and Schwarze the basophilic staining reaction can be explained on the assumption that those granules of eosinophil myelocytes which stain in indulin (indulin-aurantia-eosin staining mixture) are the youngest granules. They contain a great amount of water, and, therefore, they have an affinity for the indulin of the staining mixture since it possesses the largest molecules. After fixation at high temperatures, however, the granules give up their water and become condensed to such an extent that they no longer absorb the indulin. On account of their smaller molecules the eosin and aurantia of the mixture are still able to penetrate the granules. The change in staining reaction is, therefore, due to physical rather than chemical causes. This accounts for the similar change in staining reaction during the "ripening" of the granules which is largely a process of condensation due to the loss of water.

Pappenheim, Hirschfeld, Hesse, Benacchio, Kardos, Szécsi, and others have observed changes in the staining properties of the basophilic granules of eosinophil myelocytes. In fact, these authors

[1]) The presence of basophilic granules in eosinophil myelocytes is assured by numerous investigators among whom may be mentioned the following: Arnold, Hirschfeld, Pappenheim, Hesse, Weidenreich, Browning, Gütig, Grawitz, Blumenthal, Maximow, Downey, Benacchio, Kardos, and Ringoen.

have reported that there is transformation from the basophilic to the acidophilic type of granulation. No attempt, however, was made by these authors to give minute descriptions of the gradual changes in the staining properties of the granules during such a transformation process, and of the phenomena which accompany it.

The work of Maximow and Downey has revived our interest in the transformation theory of the eosinophil leucocyte granules of the bone marrow. Both of these authors find that the first granules are basophilic, and this they interpret as indicating endogenous origin.

III. Material, methods, and observations.

1. Bone marrow of rabbit.

Of the various methods tried for working out the life-history of rabbit eosinophil leucocyte granules, none gave more decisive results than did the method of staining marrow smears in a mixture of indulin-aurantia-eosin. These smears were fixed in Helly's fluid for a period of fifteen minutes, and then washed in running water from three to four hours The preparations were dehydrated and left in eighty per cent alcohol until the time of staining. The staining process was carried out in an electric oven which registered 40° C. This method of procedure gave excellent results for both eosinophil and special myelocytes.

For studying the life-history of eosinophil leucocyte granules, Benacchio's method of treating bone marrow smears was also tried. This consisted in making marrow smears in a salt solution, and allowing them to dry before fixation with heat or alcohol. With this method the majority of the granulocytes are distorted, their granules are swelled, and the outlines of nuclei are usually indistinct. Downey has pointed out the inadequacy of Benacchio's technique, although he reports that it will preserve the granules in the mast leucocytes of the guinea pig, as its mast granules are very resistant to harsh treatment. According to the author, Benacchio's method, however, will not preserve similar granules in the mast leucocytes of rabbit bone marrow. Summarizing the conditions described here and previously, we have to make the following objections to Benacchio's technique, and to the conclusions that he came to as a direct result of his method: 1) his technique is inadequate for the finer histological study of the eosinophils and special cells of the marrow: 2) it is absolutely inadequate for the preservation of mast granules in the mast leucocytes of rabbit marrow; 3) and according to Downey, it is not the most favorable method to use for the study of the mast leucocyte granules of the guinea pig

marrow; 4) the method is not sufficiently adequate to warrant the conclusion that the mast leucocytes of the guinea pig are "unripe" eosinophils and special cells (Downey).

Benacchio's interpretations are based upon the proposition that his technique demonstrates all the types of granulocytes in the bone marrow of rabbit and guinea pig. Such a view, however, does not hold true for the rabbit, because its mast leucocytes, on account of the extreme solubility of their granules in water, require special methods of technique, especially proper fixation.

Marrow smears of adult rabbit contain all the various forms of eosinophils which one encounters in the blood-stream, but the majority have round or slightly polymorphous nuclei. Their granules show considerable variation in morphology, for in the earlier myelocyte stages they are usually rounded (fig. 3), while in the fully differentiated cell they are spindle-shaped (fig. 4). Downey states (1915, p. 180):

"the elongation of the granules usually takes place at about the time that the nucleus becomes sharply indented on one side (fig. 17). There are some variations from this rule, which may be partly accounted for by the variation in the time of appearance of the first granules."

The size of the granules is subject to considerable variation during their developmental stages. In the fully differentiated leucocyte (fig. 4) the granules are of approximately the same size, although there is some variation in this regard. There may be similar variations in any eosinophil leucocyte of the blood-stream. The myelocytes (fig. 3) usually contain a number of granules which are larger than those of the fully differentiated cell. These variations can be readily seen on comparing figure 3 with figure 4; they are drawn at the same magnification.

The myelocyte of figure 3 shows that there may be significant variations in the size of the granules in any given myelocyte. In this particular instance, some of the granules appear as mere specks in the cytoplasm, while others, as previously pointed out, exceed in size those of the fully differentiated cell (compare fig. 3 with fig. 4). Downey, describing the granules of the myelocytes, states:

". . . as the nucleus becomes polymorphous the granules become more uniform in size, and at the same time the largest ones are reduced in size, probably by becoming more compact. Since many of the mature cells contain some small granules, we may conclude that some of the smallest granules of the myelocytes never enlarge. These small granules are probably the last ones to be formed, and have therefore not reached their full size before all further growth of granules is checked."

In the rabbit the largest granules are also reduced in size, for in

the fully developed eosinophil leucocyte most of the granules are of practically the same size; real large granules are not present. I believe that Downey's interpretation of the very small granules in the more mature eosinophil myelocytes as representing recent differentiations holds true for similar granules in the eosinophil myelocytes of the rabbit marrow.

Downey also lays great stress on the fact that the very first granules which are differentiated in the cytoplasm of an eosinophil myelocyte are always small (Downey 1915, figs. 6, 7, 27). In the interpretation of this condition I find too that shortly after the granules have appeared they tend to enlarge very rapidly. This conclusion is based on the presence of numerous small granules intermixed with others that are slightly larger, and still others that are many times larger (fig. 3), together with the observation that in the fully differentiated eosinophil leucocyte few small granules are present (fig. 4).

The significance of the conditions described are of real importance for a true understanding of the life-history of eosinophil leucocyte granules; especially since there is no evidence of degeneration of any of the myelocyte granules. One of the phenomena to be observed is that the small granules increase in size, and finally also change their configuration. This indicates that the granules undergo a process of "ripening".

There are other conditions which indicate that the "ripening" process of the granules is of a complex nature, for in addition to the changes described, one is impressed with the fact that there are other very specific ones. These relate to the changes in the staining reaction of the granules.

Many investigators believe that the basophilic granules of eosinophil myelocytes represent the youngest eosinophil granules. Weidenreich, however, states emphatically, although he admits that eosinophil myelocytes may contain basophilic granules, that where eosinophil granules appear they are characterized as completely formed elements. Grawitz also believed that there is no relation between basophilic granules and those that stain in eosin.

Hesse (1902) observed that the basophilic granules are transformed into eosinophil granules, in the eosinophils of the rabbit marow, but is not positive that the former granules represent the youngest ones.

Maximow's (1913) figures indicate that there is direct transformation of the basophilic granules into those that are oxyphilic, and that the youngest ones are basophilic. Summarizing his findings in marrow smears that were fixed in Helly's fluid, and stained in alcoholic

thionin, the following staining reactions are significant: fully differentiated eosinophil granules are stained green, while the corresponding myelocytes contain a number of granules that are colored blue, and others that are green in color. The same cells also contain granules that show intermediate staining reactions.

Downey (1914/15) believes that the fully differentiated eosinophil granules are the end product of a series of gradual, complex changes in chemical constitution, as well as in form and size, of the basophilic "unripe" granules which first appeared in the myelocyte.

Let us now consider the basophilic granules in the eosinophil myelocytes of the rabbit bone marrow. These granules show considerable variation in size; many of them are mere specks in the cytoplasm, while others are a little larger than the vast majority of the oxyphilic granules of the fully differentiated eosinophil leucocyte. Fig. 2 shows great variation in the size of the basophilic granules, as well as in those that are oxyphilic. The myelocytes of figures 2 and 3 are not of the same age, but the number of granules is approximately the same. In the myelocyte of figure 2, which is apparently the older cell, the basophilic granules are numerous and some of them very minute. However, none of its oxyphilic granules are larger than those shown in figure 3. In the latter figure, the majority of the oxyphilic granules are of uniform size.

A careful study of the myelocytes represented in figures 2 and 3 show that many of the larger granules are stained in a mixed tone, although there is considerable variation in this regard. Many of the granules are stained black, others show a tinge of red, and still others are predominantly red with only a suggestion of the indulin color (fig. 3).

That the largest granules may show intermediate staining reactions, or be predominantly oxyphilic is of real significance. It is evident, on comparing the largest round granules of the myelocyte of figure 3 with similar granules in figure 4, that even the largest granules are transformed into eosinophil granules, although they may remain large for a time. That these largest granules are subsequently reduced in size has already been considered.

Since the life-history of the eosinophil leucocyte granules in the marrow of the guinea pig is duplicated in a most remarkable manner in the rabbit, I shall refer the reader to Downey's paper for the consideration of Weidenreich's view with reference to the nuclear origin of the large basophilic granules and their subsequent degeneration.

Downey reports that basophilic granules may change their staining

reaction at any time.[1]) In the rabbit marrow, I find similar conditions. This is indicated by the fact that many of the smallest granules are acidophilic, while many of the larger ones are of a mixed tone. The smallest granules may also show a mixed tone which indicates that they change their staining reaction before reaching any appreciable size. However, they may retain their straight basophilic character for some time. In the myelocyte of figure 2 a number of the very smallest granules are already acidophilic. The basophilic granules have the same configuration as those shown in figure 3. According to Downey, "the two sorts of granules differ only in chemical constitution". In the fully differentiated eosinophil leucocyte of the marrow all the basophilic granules have been transformed into acidophil granules, and no new basophilic granules are formed.

These gradual progressive changes in the morphology and staining reaction of basophilic (indulinophilic) granules in the myelocytes of eosinophil leucocytes must be interpreted as indicative of progressive evolution on the part of the eosinophil granules.

The granules of both the eosinophil and special myelocytes are very small when they are first differentiated in the cytoplasm, and both types of granules are stained dark with indulin (indulinophilic), or basophilic with heterogenous mixtures containing a basic dye. This uniformity in size and staining reaction frequently makes it very difficult to distinguish the earliest special myelocytes from those of the eosinophil myelocytes. Maximow encountered similar difficulties in rabbit embryos, but claims that he can always distinguish the two types of myelocytes from one another in the marrow of post-natal animals. He states that in the latter the first eosinophil granules are from the very beginning brighter and coarser than are those of the special myelocytes; at first the youngest granules possess a clear basophilic quota and stain a bluish tinge, but are not metachromatic as are the granules in the special cells. In spite of these differences there are times when it is almost impossible to classify myelocytes containing basophilic granules with any degree of certainty. Maximow states that the basophilic granules in the eosinophil myelocytes can often be distinguished from those of special myelocytes by the fact that some of the eosinophil granules enlarge very rapidly "und daß man infolgedessen schon in den

[1]) Downey (1915, p. 190) states also that "the development and transformation of the granules is usually associated with very definite changes in the structure and shape of the nucleus. However, there are many exceptions to this rule. . . . These variations are undoubtedly partey due to the fact that the first granules may appear in lymphocytes of different types in which the condition of the nucleus is variable".

noch ganz granulaarmen Zellen typische, grobe, glänzende azidophile Granula neben nur sehr spärlichen feineren erblicken kann".

Downey also states that there may be considerable difficulty in distinguishing between eosinophil and special myelocytes when their granules are few in number and of small dimensions. He also finds that the granules of eosinophil myelocytes enlarge shortly after they are differentiated and that some of them change their staining reaction, while others remain basophilic for a longer period of time. The basophilic granules in young special myelocytes, on the other hand, remain basophilic for some time, and then rapidly change their staining reaction: this change involves all the granules at about the same time.

The same difficulty in determining the exact position of the earliest myelocytes was encountered in the present study. Indulin-aurantia-eosin preparations, however, were very helpful in clearing up some of these difficulties. The chief advantage of this method is that it practically eliminates the special myelocytes from our consideration, at least in the later myelocyte stages, since the granules of the special cells at no time in their evolution show any great affinity for the eosin of the triglycerine staining mixture. Most of the special cells contain dark-grayish-black granules, but in the youngest myelocytes these granules have also a slight affinity for the eosin, a condition which frequently renders it difficult to distinguish the earlelst eosinophil myelocytes from special cell myelocytes. The special granules, however, very shortly develop their pronounced affinity for the indulin to the complete exclusion of the eosin, while the granules of many of the eosinophil myelocytes become strongly eosinophilic, causing them to stain intensely with the eosin of the triglycerine staining mixture.

The myelocyte shown in figure 1 is classified with great difficulty. It is slightly younger than the myelocyte of figure 2, and of approximately the same age as that shown in figure 3. All of its granules are indulinophilic (basophilic). It seems therefore that the myelocyte in question must belong to the special cell series, since the granules of the special cells never show any great affinity for the eosin of the staining mixture during their evolution.

The inability to diagnose positively the specific type of basophilic myelocyte in every instance does not invalidate the conclusion in regard to the life-history of the eosinophil leucocyte granules. The crucial point is, that eosinophil granules are manifestations of protoplasmic activities, and that they undergo a series of progressive evolutionary processes — a "ripening" — during which they change their shape and staining reaction.

It has not occurred to any one to claim that the granules of the special cells are not of endogenous origin. It is therefore difficult to understand why the same conclusion should not apply to the granules of the eosinophil leucocytes of the bone marrow, especially when there is no indication that fragmenting erythrocytes or other haemoglobin products are in any way concerned in their origin. Haemoglobin is liberated in the bone marrow or brought into it in solution, according to Marwedel, Brass and others, but these authors have not shown that it is a contributing factor in the formation of eosinophil granules. Marwedel maintains that the pigment, which he noted in the eosinophil myelocytes, was formed from eosinophil granules, and not from haemoglobin in solution. According to Arnold, fully formed eosinophil granules may take up various extraneous substances, and among them iron. This accounts for the fact that eosinophil granules may or may not respond to the iron test.

The results of the present study warrant the conclusion, that the eosinophil leucocyte granules of the marrow are true endogenous formations. Weidenreich's conclusions, as previously stated, are based on studies of haemolymph nodes, and the tâches laiteuses of rabbit omentum following repeated intraperitoneal injections of foreign erythrocytes; consequently they are not applicable to the marrow. Gütig's results on the bone marrow of the pig are confirmatory of Downey's and the author's with reference to the endogenous origin of the eosinophil granules. In the lymph nodes, however, Gütig states that the eosinophils appear to be of different character from those in the marrow and blood. He believes that their granules are phagocytosed fragments of erythrocytes.

The technique — Helly fixation, Ehrlich's triglycerine staining mixture — used for the present study, proved to be very good for bringing out the real differences between the basophilic myelocytes of eosinophils and those of mast leucocytes. Maximow has previously pointed out these differences in the marrow of the rabbit, but it may be well to emphasize them again, especially since I have used different fixation methods.

It will be recalled that Pappenheim's students claimed that real mast leucocytes in the blood-stream of the rabbit are not true granulocytes. Benacchio believes that the basophilic myelocytes in rabbit and guinea pig marrow are not the precursors of mast cells, but that they represent "unripe" stages of eosinophils and special cells. Pappenheim-Szécsi came to similar conclusions from their findings in the blood of rabbits which had been injected with saponin and nucleic acid.

Maximow, however, has pointed out that the supposed intermediate stages — "unripe" eosinophils in various stages of development which they claimed lead over to cells, which were identical with the true mast leucocytes of the blood, were artifacts due to inadequate fixation.

Marrow smears, that are fixed in Helly's fluid and stained with Ehrlich's triglycerine staining mixture, contain no other types of basophilic myelocytes excepting those that show changes in their staining reaction at some time or other during their life-history. Such granules, however, are not mast granules, for the latter have never been known to change their staining reaction. We must, therefore, give Benacchio credit for the correct interpretation of his preparations when he states that the myelocytes which contain basophilic granules are young "unripe" eosinophil and special leucocytes. He failed to find mast myelocytes, because his technique was inadequate for the preservation of their granules.

The writer has found the acetone-lucidol method of Szécsi (1913b) to be the most satisfactory technique for the preservation of rabbit mast granules. When fixed with this method and stained with the May-Giemsa mixture, marrow smears are seen to contain mast cells whose granules are stained an intense bluish-black color, while those of eosinophil and special myelocytes are of a reddish tinge. Figs. 5 and 6 which are drawn from preparations of this kind portray the real differences in the character of these granules. The former represents a mast myelocyte, and the latter either an eosinophil or special myelocyte. The sharp contrast in the staining reaction of the mast myelocyte granules is so pronounced and characteristic that every mast myelocyte is easily separated from the eosinophil or special myelocyte series. This is contrary to the statements of Weidenreich, who believes that if all of the granules of the eosinophils and special cells were basophilic when first formed, it would be impossible to distinguish their myelocytes from the basophilic myelocytes of mast leucocytes. I have found, however, that mast granules can always be distinguished from the granules of other basophilic myelocytes, provided that the proper methods of fixation have been applied to the marrow.

2. Bone marrow and haemolymph nodes of sheep.

Thus far, the present account has not been concerned with the origin of the eosinophils outside of the bone marrow. The question of whether the granules of the tissue eosinophils pass through similar changes during their development, as has been described for the eosino-

phils of the marrow, or whether they are of exogenous origin remains open.

Barbano's (1914) recent study of the subject indicates merely that the granules of the tissue eosinophils may be quite variable in their affinity for eosin. These variations, however, do not seem to be associated with any particular stage in the development of these leucocytes, and there is nothing which indicates that those granules which possess the least affinity for the eosin are the youngest ones.

Gütig (1907) claims that there are two types of eosinophils, haematogenous and histogenous. He considers the granules of the former type as true endogenous formations, while those of the latter are phagocytosed fragments of erythrocytes.

Weidenreich is the first investigator to claim that eosinophils in haemolymph nodes of sheep and rat are formed from lymphocytes which have phagocytosed erythrocytes or their fragments. Ascoli, however, does not believe that phagocytosed erythrocyte fragments are related to eosinophil granules. He states that he observed very few intermediate stages which would justify such an interpretation.

If under normal conditions eosinophils are formed in haemolymph, nodes of sheep, it should not be a difficult problem to determine their origin in these structures, especially if the granules of these cells are related to haemoglobin or its dissociation products, for it is indeed quite generally admitted that blood destruction goes on very actively in haemolymph nodes of the Ungulata.

Sheep marrow was studied first in order to determine whether its eosinophils pass through the same progressive changes as those described for the rabbit. The smears were fixed in Helly's fluid and stained in triglycerine and in Dominici's mixture. About twenty-four animals were used in this study.

It is evident from this material that the eosinophils of the sheep marrow pass through similar developmental stages as those which have already been described for the eosinophils of the rabbit's marrow. The first granules to appear are small, but with the increase in number, there is also a marked increase in the size of some of them. This variation in size is seen particularly well in the earlier myelocyte stages. In the older myelocytes there is more uniformity in their dimensions, although many of these cells contain a few granules which are larger than those of the fully differentiated eosinophil leucocytes. In the latter, the granules are spherical and of uniform size. No change in shape was noted in any of the stages.

Variations in the staining reaction of the granules, similar to those

described for the rabbit, were also noted in the sheep marrow. These differences are especially evident in those myelocytes which contain granules showing considerable difference in size. With Dominici's staining combination some of these granules are basophilic, some are acidophilic, while others are polychromatophilic and have evidently absorbed both the basic and acid components of the mixture. With the triglycerine mixture the results are the same: the indulin taking the place of the toluidin blue of the Dominici stain. Since all of these variations in staining may be seen within the same cell, and since there is no evidence for the degeneration or disappearance of the basophilic or polychromatophilic granules, one must conclude, as in the rabbit, that the chemical and physical constitution of the basophilic granules is so changed that they become oxyphilic. This, together with the change in size noted above, indicates that many of the granules of the eosinophilic leucocytes of the sheep pass through a gradual "ripening" process as do those of the marrow of the rabbit.

The sheep differs from the rabbit, however, in that the basophilia of the granules is not so pronounced as it is in the case of the rabbit. Basophilic granules which have absorbed none of the acid dye are rare in the sheep, although polychromatic granules are fairly numerous. Many of the first granules to appear in the early promyelocytes are decidedly oxyphilic and there are many promyelocytes and myelocytes in which all of the granules are oxyphilic. However, the variation in size of the granules is seen in these cells, as well as in those containing basophilic and polychromatophilic granules, which seems to indicate that the process of "ripening" is taking place in both types of cells. Even in the rabbit and guinea pig it is difficult to prove that all of the granules pass through a basophilic stage, but in these animals the basophilic quota of the granules is decidedly more pronounced than it is in the sheep.

This condition in the sheep probably accounts for Weidenreich's statement that the eosinophil granules possess all of their specific and fully differentiated characters when they first appear. However, the gradual change in size of the granules, many of them even exceeding the dimensions of those of the fully differentiated cells, together with the basophilic and polychromatophilic condition of the granules of many of the eosinophil myelocytes, indicates that even in the sheep the granules of the eosinophil leucocytes pass through a gradual "ripening" process.

The reduced basophilia of the granules of the sheep may possibly be accounted for by the fact that in this animal the homoplastic form

of leucocyte production shows decided predominance over the heteroplastic development. The preparations show that non-granular "stem" cells are not numerous; early promyelocytes with only a few granules in their cytoplasm are correspondingly rare; myelocytes are exceedingly numerous, but they are of the later myelocyte and metamyelocyte stages and they are packed with granules. Correlated with these findings there is also marked evidence of mitosis.

It is possible that the age or condition of the animals might have influenced the form of leucocyte production, as is indicated by the case of a rabbit with a marked leucocytosis due to the presence of a graft of foreign tissue which did not "take". The marrow of this animal shows little evidence for heteroplastic development of any type of leucocyte. Very few of its eosinophil myelocytes contain basophilic granules, and there are very few "myeloblasts" present. This marrow appears very different from that of a normal young rabbit in which the non-granular lymphoid cells and eosinophil promyelocytes with numerous basophilic granules, are abundant.

Weidenreich (1911) admits the presence of basophilic granules in eosinophil myelocytes, but he claims that if all of these granules were basophilic when first formed, the basophilia should be particularly pronounced during homoplastic regeneration. The writer is unable to see the force of this argument. The facts seem to be just the reverse, for the material shows that the basophilic constituent of the granules is more evident when the heteroplastic form of regeneration from non-granular cells is pronounced.

Nothing was seen in the sheep marrow which could be interpreted as indicating a relationship between eosinophil granules and haemoglobin. All the observed facts favor the view that the granules are endogenous differentiations resulting from the protoplasmic activity of the cells which carry them.

On account of the claims of Weidenreich, Gütig, Badertscher, and others, it might be assumed that the eosinophils of the haemolymph nodes of sheep represent a type of leucocyte with an origin very different from that of the blood eosinophil, which is usually believed to be derived exclusively from the bone marrow. Studies which might determine the origin of the numerous eosinophil leucocytes of these organs are, therefore, of special interest, especially when preceded by studies on the regeneration of the eosinophils in the marrow of the same animals.

The writer used retroperitoneal hemal nodes from the lumbar region

of some two dozen animals; the material being removed and fixed in Helly's fluid immediately after the sheep were killed.

Great numbers of eosinophils are present in sections. Many of them are evidently old cells, for their nuclei stain deeply, and that many of these leucocytes degenerate within the node is shown by the presence of numerous small heaps of eosinophil granules. In their shape and staining reaction they are identical with the granules found in typical eosinophil leucocytes.

Many eosinophil leucocytes are developed in haemolymph nodes, for numerous mononuclear forms are present. They are variable in size: their nuclei are of the myelocyte type. The number of granules in the myelocyte cell-body is variable, in some of them the cytoplasm is filled with granules, while in others small cytoplasmic areas contain none.

These granules vary in size, the smaller ones represent recent differentiations. That the small ones increase in size is shown by the fact that in the fully differentiated eosinophil leucocyte all of the granules are larger and of practically uniform size.

During their progressive evolution, the granules exhibit changes in their staining reaction. In the early myelocyte stages most of the large granules are of a reddish color with the triglycerine mixture, while those of the fully differentiated eosinophil leucocyte are of a pinkish color. The small granules in the myelocytes, as well as a number of the larger ones, are basophilic, indulinophilic, or polychromatophilic. The intermediate tints show that there is progressive transformation from the dark basophilic condition to those that are of mixed character. The oxyphilic condition manifests itself in the later myelocyte stages.

These facts show that the granules of the eosinophil leucocytes, in haemolymph nodes, are also true endogenous formations. In the myelocyte stages the developing granules are variable in size and staining reaction, which indicates that there is progressive evolution on the part of these granules. Therefore, they are not, as Weidenreich claims, derived from haemoglobin or its products.

Sections of a great many nodes show that there is considerable variation in the number of erythrocytes which they contain. That there is extensive degeneration of these cells, as claimed by Weidenreich, is not evident from my preparations. Undoubtedly there is some degeneration, for frequently red cells are seen that stain very faintly, and many of these are of unusual shapes. Erythrocyte fragments were observed, but they were never numerous.

A few erythrophages could occasionally be found, but erythrophagocytosis was not a usual condition. Phagocytosis of erythrocytes and erythrocyte fragments by the polymorphonuclears, as reported by Meyer (1914), was never observed. Phagocytosed fragments were never noted in any type of cell.

A possible explanation for the presence of large numbers of eosinophils in the haemolymph nodes will be given later in connection with the general discussion.

The results of this study show that the general life-history of the eosinophils in haemolymph nodes of sheep is a duplication of that of the eosinophils in the bone marrow.

3. Bone marrow and thymus of pig.

Since Badertscher chose thymus material from 125 mm pig embryos, the writer has selected his material from embryos of a similar length. The marrow was obtained from adult pigs, and it will be considered first.

Bone marrow smears contain great numbers of eosinophil myelocytes, and their corresponding leucocytes. It appears that all of the myelocyte granules are differentiated at one time, for their cytoplasm is filled with granules. Furthermore, they are of uniform size and this indicates that no gradual differentiation is taking place.

Many of the early myelocytes contain dark (indulinophilic) granules only, but they are not basophilic, as they fail to take the basic component of a heterogeneous staining mixture. With the triglycerine mixture some of the granules in the older myelocytes take on a mixed tint, while others lead over to those that are oxyphilic. The intermediate colors show that the progression of changes is from the blue-black indulin color to eosin.

Sections of thymus show great numbers of eosinophil leucocytes, but most of them are of the mononuclear type. According to Badertscher these mononuclear forms are derived from lymphocytes that have taken up erythrocyte degeneration products. He finds lymphocytes with only a few granules in their cytoplasm, and, therefore, this seems..... "to suggest that some eosinophile cells are simply lymphocytes ingested with the débris of degenerated erythrocytes".

According to Hartmann (1915) and Weill (1913), however, Badertscher's findings do not hold true for the mononuclear eosinophils of the thymus of rabbit, rat, and homo. Hartmann states specifically that these cells are always filled with granules, and the figures given by Weill show similar conditions.

Badertscher claims that degeneration of free erythrocytes gives origin to groups of free eosinophil granules. Hartmann (1915, p. 173), however, states specifically that she saw no fragmentation of red cells in the thymus of embryo rabbits, and the writer has seen no evidence of it in pig thymus.

That lymphocytes are capable of differentiating granules in their cytoplasm is shown by Downey and Weidenreich (1912), Dominici (1909), Weill (1913), Barbano (1914) and Herzog (1915). None of these authors — with the exception of Weidenreich — claim that the eosinophil granules are related to haemoglobin or its products.

Pig thymus material contains great numbers of myelocytes that are differentiating eosinophil granules. Maximow (1912) and Hartmann (1915) have observed similar myelocytes in their investigations on developing thymus. The latter finds that these myelocytes may be very numerous in rabbit thymus, but she does not regard them as autochthonous elements because it is not evident that their granules are differentiated gradually in non-granular cells.

Figure 7 represents a mononuclear form with typical myelocyte characters. The peripheral portion of the cell as well as a small area about the nucleus is still comparatively free of granules. It is evident that most of the granules are first differentiated in the main body of the myelocyte, while the peripheral portion contains very few. The peripheral granules represent recent differentiations, for they are slightly irregular in shape. This conclusion is based on the observation that in the fully differentiated eosinophil leucocyte the granules are all of approximately the same shape.

These granules are not at any time basophilic, but with the triglycerine staining mixture they may exhibit indulinophilic properties. In this respect they are identical with the granules in the eosinophil myelocytes of adult pig marrow.

In the myelocyte of figure 7 several of the peripheral granules are indulinophilic, while a few others take on an intermediate tone between the indulin and eosin of the triglycerine mixture. These granules, that have an intermediate color, lead over to those that have a decided affinity for the eosin of the stain.

The great majority of the granules (fig. 7) are very dense, and rather indistinct in outline. Their density is responsible for their deep red color. In the fully differentiated eosinophil leucocyte the granules are less dense and they have a clearer red tinge. Their clear color may also be associated with the fact that they are now mature and have, therefore, completely lost their indulinophilic properties.

These observations, together with those previously described, prove that the granules pass through changes in their staining reaction as they approach maturity.

In general the results on the thymus agree with the findings of Maximow, Weill, and Hartmann, although these investigators have not made a special study of the origin and real character of the eosinophil leucocyte granules.

4. Peritoneal fluid, omentum and subcutaneous tissue of experimental animals.

Weidenreich claims that local development of eosinophils takes place in the omentum as a result of intraperitoneal injections of foreign erythrocytes; the eosinophil granules being derived from the haemoglobin of these erythrocytes. He also believes that under normal conditions the granules of these cells have a similar origin[1] [2]). He states emphatically that „Eine andere Bildung der Granulationen ist bisher nicht beobachtet worden, besonders nicht eine allmähliche Differenzierung aus einem nicht granulären Plasmaleib".

Downey (1914/15) and the author, however, have proved that Weidenreich's theory does not hold true for the eosinophil granules in the eosinophil leucocytes of the bone marrow, and the latter finds that the theory is also inapplicable to similar granules in the eosinophils of haemolymph nodes.

According to Weidenreich there are three possibilities for the origin of eosinophil granules: 1) from erythrocyte degeneration products; 2) from phagocytosed intact erythrocytes; 3) and possibly from haemoglobin in solution which is phagocytosed, worked over and deposited in the form of granules.

In my experimental studies, all of the above mentioned possibilities have been given careful consideration. The following experiments were performed: single intraperitoneal injections of erythrocyte suspensions; repeated intraperitoneal injections of erythrocyte suspensions at intervals of eight or twelve days; single intraperitoneal injections of haemo-

[1]) Anat. Rec. 1910, vol. 4, p. 327, „Die eosinophilen Granula der Säugetiere sind als exogene Plasmaeinlagerung zu bezeichnen und zwar als hämoglobinhaltige Teile, größtenteils von Erythrozyten herrührend, die durch hämolytische Vorgänge zerstört, oder in toto phagozytiert wurden."

[2]) Anat. Anz., 1901—1902, vol. 20, p. 197, „Die eosinophilen Leukozyten sind also nichts anderes als sogen. Lymphozyten, welche die durch den Zerfall roter Blutkörperchen entstehenden feinen Trümmer in ihrem Plasmaleib aufnehmen, wobei ihr Kern in die polymorphe Form übergeht."

globin and daily injections repeated for several days; repeated intraperitoneal injections of haemoglobin at eight or twelve day intervals; repeated intraperitoneal injections of haemoglobin and erythrocyte suspensions at intervals of eight or twelve days; repeated intraperitoneal injections of egg-white at similar intervals; and subcutaneous injections of either a suspension of erythrocytes or a straight haemoglobin solution.

Rat blood was utilized in all the erythrocyte and haemoglobin experiments. The blood from a freshly killed specimen was defibrinated and placed in a centrifuge tube. The tube was filled halfway with physiologic salt solution and a mixture effected by means of a glass rod. The contents of the tube were centrifugalized long enough for the erythrocytes to fall to the bottom of the tube, when the supernatant fluid and layer of leucocytes were pipetted off and fresh saline again mixed with the cells. The centrifugalizing process and washing with saline were repeated until the supernatant fluid was free from serum. After the last washing the supernatant fluid was pipetted off and the packed erythrocytes were diluted with three volumes of saline. Two ccm. of this suspension were used in all intraperitoneal and subcutaneous injections.

The haemoglobin solution was obtained by laking washed corpuscles. After haemolysis had taken place, the solution was placed in a centrifuge tube and centrifugalized. One cubic centimeter of the clear fluid containing the hemoglobin was injected either intraperitoneally or subcutaneously.

Fifty animals were used in these studies. The great majority of these were guinea pigs, although a number of rabbits were also used. As far as results are concerned, however, there were no differences in these animals.

The problems to be solved required that most of the work be done on the peritoneal fluid, omentum, and subcutaneous tissue. These tissues were treated by a variety of methods. Ordinary dry smears of peritoneal fluid were stained with either Wright's or Johnson's blood stain (a modification of the Romanowsky method), and moist smears, prepared according to Deetjen's method, with Giemsa. Omentum and subcutaneous preparations were prepared according to Maximow's method. This consists in spreading the omentum over the mouth of a glass tube; the subcutaneous tissue is spread on cover-slips by means of needles. Helly's fixation and Dominici's stain were used for these preparations.

The results obtained with single intraperitoneal injections will be

considered first. The results of a number of these injections are shown in table 1.

Table 1.

Showing the results of single intraperitoneal injections of foreign erythrocytes.

Animal	Sus. Er.	Per. Fluid	Blood
G. P.	Sus. Er.	2 hrs. after injection eosinophils contain red cells. Macrophages also contain red cells.	
		10 hrs. after injection polynucl. are numerous.	
		22 hrs. after injection no increase in No. of eosinophils.	22 hrs. after injection no increase in No. of eosinophils.
G. P.	Sus. Er.	Eosinophils and macrophages are slightly phagocytic for red cells.	16 hrs. after injection no increase in No. of eosinophils.
G. P.	Sus. Er.	Same conditions as in previous animals.	18 hrs. after injection eosinophil count unchanged.

The data above indicates that the eosinophil leucocytes are not increased in numbers after single erythrocyte injections, and these results agree with those of Stschastnyi for the eosinophils of the peritoneal cavity. The fact that the foreign erythrocytes are phagocytosed by both macrophages and eosinophils corresponds in general to what is known of the phagocytic activities of these cells.

Weidenreich, Stschastnyi, and Sternberg made repeated injections of foreign erythrocytes — at regular intervals — into the peritoneal cavity. The results of a number of similar injections are shown in table 2.

From table 2 it is seen that the eosinophil leucocyte count is increased after a second intraperitoneal injection of foreign erythrocytes. This applies not only to the eosinophils of the peritoneal cavity, but also to those of the blood-stream. In three of the four animals — results tabulated above — the eosinophils were most numerous in the blood and peritoneal cavity after the fifth injection of erythrocytes. In the fourth animal, guinea pig no. 19, however, they were very numerous after the third injection.

Single intraperitoneal injections of haemoglobin have practically the same negative results, so far as the eosinophils are concerned, as do single injections of erythrocyte suspensions (table 1), and repeated daily intraperitoneal injections of haemoglobin extending over several days give similar negative results.

Table 2.

Showing the increase in the number of eosinophils in the blood and peritoneal cavity after repeated intraperitoneal injections of erythrocyte suspensions; 8—12 days intervened between injections.

G. P. no.	Untreated		First Injection			Second Injection			Third Injection			Fourth Injection			Fifth Injection			Remarks
	Per. F.	Blood	Time [1]	Per. F.	Blood	Time	Per. F	Blood	Time	Per. F.	Blood	Time	Per. F.	Blood	Time	Per. F.	Blood	
16	Few	6,8%	24 Hr.	No increase	No change	24 Hr.	Slight incr.	11,4%	24 Hr.	Numerous	24%	24 Hr.	Abundant	14%	24 Hr.	Very abundant		No local development of eos. in omentum.
						48 Hr.	Increase		48 Hr.	More numerous		48 Hr.	Abundant		48 Hr.	Very abundant	17%	
						96 Hr.	Decrease											
17	Few	7%	24 Hr.	No increase	7.9%	24 Hr.	Slight increase	10%	24 Hr.	Numerous	15%	24 Hr.	Abundant	–	24 Hr.	Marked increase	L. 38% L. M. 9,2% Mast. 2,2% Eos. 20,2% Poly. 30,4%	No local development of eos. in omentum.
															48 Hr.	ditto	19%	
19	Few	7,9%	24 Hr.	No perceptible increase	18,1%	24 Hr.	Slight increase	12,2%	24 Hr.	Numerous	L. 37,5% L. M. 8% Poly. 33,2% Eos. 19,2% Mast. 2,1%	—	—	—	—	—	—	Animal was killed after 3. inj. Slight local development of eos. in omentum. Per. fluid contains mononuclear eos.
						48 Hr.	Marked increase		48 Hr.	Very numerous	L. 40,1% L. M. 9% Poly. 29,1% Eos. 20,6% Mast. 3,1%							
20	Very few	7,8%	24 Hr.	No apparent increase	8,2%	24 Hr.	Slight increase	15,5%	24 Hr.	Numerous	16,8%	24 Hr.	Abundant	19%	24 Hr.	Very abundant	22%	No local development of eos. in omentum.
						48 Hr.	Slight increase		48 Hr.	Very numerous	17,9%	48 Hr.	Very abundant		48 Hr	Extremely abund.	24%	

[1]) The word time indicates the number of hours that elapsed after the last injection and the examination of the blood and peritoneal fluid.

Repeated intraperitoneal injections of hemoglobin, however, give very different results provided that a number of days intervene between injections. The data on the results of these experiments are an exact duplication of those obtained with repeated injections of erythrocytes (table 2). In most of the haemoglobin experiments, however, few of the animals survived beyond the third intraperitoneal injection.

Two intraperitoneal injections of a mixture of an erythrocyte suspension and haemoglobin, given at definite intervals, increased the number of eosinophils in the peritoneal cavity and blood-stream. Since the guinea pigs usually died immediately after a third injection, it was impossible to determine to what extent this injection would have accentuated the number of eosinophil leucocytes.

Repeated intraperitoneal injections of egg-white, which were also made at definite intervals, resulted in a marked increase in the number of eosinophils. These results are identical with those obtained after repeated injections of erythrocyte suspensions, haemoglobin, and a mixture of haemoglobin and an erythrocyte suspension. The results of the egg-white injections are given in table 3.

In these experiments the eosinophil leucocytes were most numerous after the fifth injection of egg-white. In guinea pig no. 10, however there was a marked increase in the number of these cells after the second injection.

In tables 1, 2, and 3 no attempt has been made to include all the animals that were used in the various types of experiments. Since the results were so strikingly uniform in all cases, it did not appear necessary to give more than a few examples of these experiments.

According to Weidenreich, the increase in the number of eosinophils in the peritoneal cavity is due to the breaking up of erythrocytes into fragments which are subsequently phagocytosed by lymphoid cells whose nuclei finally become bilobed and similar to the nuclei of the eosinophils of the blood-stream. These fragments remain in the cytoplasm as the eosinophil granules. Stschastnyi claims that the foreign red cells are first phagocytosed intact and then broken into granules. Weidenreich believes that this process may also take place, and that the foreign red cells, after being acted upon by haemolysins, may give up their haemoglobin. The free haemoglobin is taken up by lymphoid cells and deposited in their protoplasm as typical eosinophil granules.

The peritoneal transudation of the normal guinea pig contains numerous small lymphocytes, large lymphocytes, macrophages, and a variable number of eosinophils. Mast cells and polymorphonuclear leucocytes are not present. Szécsi (1912) claimed that he was unable to find

Table 3.

Showing the increase in the number of eosinophils in the blood and peritoneal cavity after repeated intraperitoneal injections of egg-white; 8—12 days intervened between injections.

G. P. no.	Untreated		First Injection			Second Injection			Third Injection			Fourth Injection			Fifth Injection			Remarks
	Per. F.	Blood	Time	Per. F.	Blood	Time	Per. F.	Blood	Time	Per. F.	Blood	Time	Per. F.	Blood	Time	Per. F.	Blood	
9	Few	6%	24 Hr.	No increase	6,9%	17 Hr.	Increase	12%	24 Hr.	Increase	13,9%	24 Hr.	Numerous	14,6%	24 Hr.	Abundant	16%	No local development of eos. in omentum.
						22 Hr.	Increase	14%	48 Hr.	Increase		48 Hr.	More numerous		48 Hr.	Very abundant		
10	Few	7.2%	24 Hr.	No change	7,5%	25 Hr.	Increase	19,2%	—	—	—	—	—		—		—	Animal was killed 78 hrs. after 2nd inj. Bone marrow hyperplastic.
						32 Hr.	Marked increase	20%										
						78 Hr.	Less marked	8%										
11	Few	7,6%	24 Hr.	No change	No change	23 Hr.	Increase	12,4%	—	—	—	—	—	—	—	—		Animal died shortly after 3rd injection.
						40 Hr.	Marked increase.	15,6%										
12	Few	7%	24 Hr.	No change	No change	24 Hr.	Increase	12%	24 Hr.	Increase	14,9%	24 Hr.	Marked increase		24 Hr.	Abundant	18%	No local development of eos. in omentum.
						72 Hr.		14,3%				48 Hr.	Abundant	16%	48 Hr.	Very abundant	21%	
13	Few	7,3%	24 Hr.	No change	7,9%	24 Hr.	Increase	11,4%	24 Hr.	Increase	13%	48 Hr.	Marked increase	15,8%	48 Hr.	Marked increase	18,3%	Slight local development of eos. in omentum.

any eosinophils. Stäubli (1905), on the other hand, believes that the guinea pig normally possesses great numbers of these cells. Szécsi and Ewald (1912/1913), however, demonstrated parasites in the liver of all guinea pigs that showed peritoneal eosinophilia, and therefore, the former author believed that Stäubli's animals were similarily infected. Rackemann (1915) reports that the average number of eosinophils for 22 guinea pigs was 14,173 %. According to Rackemann, "there is no fixed, normal percentage relationship between the types of cells in the peritoneal fluid of normal guinea-pigs".

The eosinophils of the peritoneal fluid are identical in all respects with those of the blood-stream; this applies to the size and shape of their granules and the configuration of their nuclei. Schott (1909, p. 172) has previously emphasized these morphological similarities. He, however, states that the majority of the eosinophils of the peritoneal cavity are of the mononuclear type. Mononuclear forms have also been reported by Weidenreich (1907, p. 51). However, they are not always present for the writer finds no similar forms in numerous guinea pigs.

After a single interperitoneal injection of a suspension of erythrocytes (2 ccm.) the peritoneal transudate presents a different picture. Smears contain numerous small lymphocytes; the macrophages and eosinophils are phagocytosing the foreign red cells. Ten to twenty-two hours after the injection there is a marked polymorphonuclear leucocytosis, but there is no increase in the number of eosinophils (table 1). Forty-eight hours after a single injection the phagocytosed red cells have completely disappeared within the macrophages and eosinophils, as shown by the large vacuoles which these cells contain. Schott is therefore correct when he states that the phagocytosed red cells are completely digested.

Phagocytosis is very pronounced after repeated intraperitoneal injections of foreign erythrocytes, provided that the second injection is given 8—12 days after the first injection; subsequent injections may be made six or ten weeks later. Following the second, third, or subsequent injections, erythrophagocytosis is by no means confined to any particular cell-type, but is especially pronounced in the large macrophages. Many of these erythrophages contain only a few ingested red cells, while others are gorged with them, and still others contain erythrocytes and their dissociation products. A number of these features are shown in fig. 8; the smear from which this cell was drawn was treated according to Deetjen's method and stained with Giesma. With this technique, free erythrocytes are of a distinct green wish color.

Stschastnyi figures cells similar to the one represented in fig. 8,

and claims that after the repeated injections of foreign erythrocytes these cells, i. e., the erythrocytes, are usually phagocytosed and subsequently, during their digestion, the haemoglobin diffuses out and appears in the form of granules in the protoplasm of the phagocyte. In view of these claims, the macrophage of fig. 8 is worthy of careful study. Its cytoplasm is filled with numerous vacuoles which vary greatly in size. Many of the small ones contain a single minute granule, others contain none, and in still others there are a few very small granules which lie in close proximity to the peripheral portion of the vacuole.

The large granules, which are green in color, represent freshly phagocytosed erythrocytes, for they stain in the same manner as the free erythrocytes. A single phagocyte frequently contains six to twelve of these cells. Whether the small clear space so frequently seen about phagocytosed erythrocytes represents a small amount of fluid which is taken in with them, as Lewis claims (1903), or whether vacuolation is a process in the destruction of the erythrocytes, as Schumacher (1899), believes, is difficult to determine. It seems, however, that there are elements of truth in both views.

The small granules, previously mentioned in connection with the cytoplasmic vacuoles, vary considerable in size. Many of them are mere specks in the cytoplasm while others are slightly larger. There is also slight variation in their staining reaction as is indicated by the differences in the tinctorial properties of these granules.

According to Weidenreich, these granules may be derived from phagocytosed erythrocytes, and that this is true is evident from the cell illustrated in fig. 8. It is apparent that the two large vacuoles represent the remains of phagocytosed red cells. The uppermost vacuole shows three very small granules along its lower peripheral border. During the destruction of the erythrocyte — which cell subsequent to its phagocytosis occupied the now vacuolated area — haemoglobin has evidently diffused out into the cytoplasm of the phagocyte. In this connection, Lewis states (1903, p. 34):

"The first action of a phagocyte upon a red cell is probably . . . . in the direction of liberating its contents, either by absorption or rupture of the cell membrane; in either the result is the formation of a spherical mass of haemoglobin. Several globules of haemoglobin may run together to form a larger mass, or, as more frequently occurs, some of the original globules break up into several smaller ones, any of which may take on the spherical form . . . .".

The findings in other cells, however, lead one to believe that the

destruction of phagocytosed red cells does not always result in the formation of granules, especially when cells similar to the one shown in fig. 9 are noted. Here there is present only one erythrocyte, but the protoplasm of the phagocyte is studded with small granules. There is considerable variation in their size, some of them are very minute while others are rather large.

With Johnson's or Wrights's stain all of these granules stain a pink color, and this applies also to the phagocytosed red cells. The peculiar staining reaction obtained when Deetjen's method is used, followed by staining the preparation with Giesma, shows that freshly phagocytosed red cells, as well as similar free cells, stain a green color. On the other hand, the small granules are of a lilac color, although a number of them may be green (fig. 8), while others may be of intermediate colors (fig. 9). Hoppe-Seyler claims that in the erythrocyte the haemoglobin is present in combination with some other substance. Possibly this explains the difference in the staining reaction of phagocytosed erythrocytes and the small variously colored granules. On the other hand, possibly the osmic acid which is used for fixation in Deetjen's method acts upon the lipoid membrane of the erythrocytes, and, therefore, these cells stain differently than do the small granules. Again there is another possibility, and it apparently offers the correct solution for these differences in staining reaction. Phagocytes (figs. 8 and 9) have been noted containing freshly ingested erythrocytes which are colored green, besides the smaller granules which are variable in their tinctorial properties. Cells presenting these features are very common. Another common phenomenon is the phagocytosis of an intact erythrocyte which, however, is not of a green color, but of a faint lilac tint. This is shown in fig. 10, which represents an eosinophil leucocyte. The staining reaction of the erythrocyte indicates that it is undergoing degeneration, for, as mentioned several times previously, free erythrocytes and freshly ingested red cells are colored green with Deetjen's method (Giesma stain). From fig. 10, therefore, it is evident that a phagocytosed erythrocyte undergoes a change in staining reaction during its degeneration (compare fig. 10 with fig. 11).

The small granules, irrespective of their staining reactions (figs. 8, 9, and 11), represent free haemoglobin which has been phagocytosed and deposited in the form of granules, proof for their haemoglobinous origin will be presented at a subsequent time. Other granules, however, as previously pointed out, may be derived from the dissolution of phagocytosed erythrocytes (fig. 8); proof for their erythrocytic origin will also be given subsequently.

A superficial examination of the cells shown in figs. 8 and 9 might lead one to suspect that Weidenreich is correct when he states that eosinophil granules are formed from erythrocytes or their dissolution products. If his theory is correct it should not be difficult to prove that the granules resulting from degenerating red cells are identical with the granules of the eosinophil leucocytes of the blood-stream. According to Stschastnyi, they are not of the same character, although he maintains that the granules of the eosinophils are derived from erythrocytes during their degeneration.

It is evident from the cells of figs. 8 and 9 that the haemoglobin granules lie in vacuoles, some of which may contain several granules (fig. 9). Within a single vacuole there may be pronounced differences in the staining properties of these granules. These differences have already been mentioned, but it is necessary to emphasize them again in this connection, for if these slight differences in staining reaction indicate that haemoglobin granules are changed physically and chemically, as Weidenreich claims, during their transformation into real eosinophil leucocyte granules, one would expect to find intermediate stages in the process. That the character of the haemoglobin granules is changed is indicated by the presence of numerous granules which are of different colors. The variation in the staining reactions of these granules are similar to those already described for phagocytosed erythrocytes. In the cell of fig. 8, there are several small green granules, while in the cell shown in fig. 9 there are several granules of practically the same size, but of varying shades of color. Some of these are of a pale green color, others are of a faint lilac tint, and still others are of a decidedly lilac color. The cell of fig. 11 contains an erythrocyte and only one haemoglobin granule; the latter in its color characters is intermediate between the large phagocytosed, faintly lilac colored erythrocyte of fig. 10 and the predominantly, lilac colored granules of the cell shown in fig. 9. All of the lilac colored granules show no subsequent changes in their color characters, therefore, if these granules represent eosinophil leucocyte granules, they should exhibit the same staining properties as real eosinophil leucocyte granules.

On comparing the granules in the cells of figs. 8 and 9 with those shown in figs 10 and 11, it is apparent that haemoglobin granules are of very different character from that of real eosinophil leucocyte granules. The Deetjen method is not the most favorable technique for the demonstration of eosinophil leucocyte granules (figs. 10 and 11), but even with this technique there can be no question about the difference in character of eosinophil and haemoglobin granules. When fixed

by this method and stained with Giesma, the former are stained a pink color, while the latter are of different lilac colors. Never are there any similarities in the staining reaction of the two types of granules. Furthermore, no intermediate stages between these granules were ever observed. Stschastnyi admits that he never saw intermediate forms, and Sternberg makes a similar statement.

The fact that the haemoglobin granules lie in vacuoles (figs. 8 and 9) would indicate that they are gradually undergoing a process of digestion. Some of these vacuoles contain a rather large granule, others several small ones, and still others contain just a trace of a granule. That the granules are finally completely digested is indicated by the presence of numerous vacuoles without the least evidence of any granulation.

The observations which have just been described are sufficient to lead one to conclude that Sternberg is correct when he states that eosinophil leucocyte granules are not derived from erythrocyte products. The fact that free erythrocytes and free haemoglobin may be phagocytosed and subsequently completely digested is analogous to the ingestion and digestion of food by an ameba.

Striking results are obtained after repeated intraperitoneal injections of haemoglobin. One ccm. of haemoglobin was used in these injections, and 8—12 days intervened between injections as in the erythrocyte experiments. The results obtained with the haemoglobin are confirmatory of those previously described in connection with the erythrocyte experiments. The reasons for these analogous results will be discussed subsequently.

The cells shown in figs. 12, 13, 14, 15, and 16 were drawn from peritoneal smears of guinea pigs. All of these smears were stained with Johnson's staining mixture. The animals had repeated intraperitoneal injections of haemoglobin which were given at 8—12 day intervals. Very few of these animals lived beyond the third injection.

It is evident from these preparations that the same phagocytic activities are going on as after the repeated injections of erythrocytes, although the picture is more striking. The variation in the size of the haemoglobin granules is pronounced. There is considerable variation after erythrocyte injections, but it is not marked. Fig. 12 shows a greater variation in the size of the haemoglobin granules after haemoglobin injections. Some of these granules are mere specks, others are somewhat larger, and still others are large haemoglobin balls. Most of the granules are of a pinkish color, although there are a few that are of a deep red color. The dark colored ones, in all probability, repre-

sent intermediate stages in the formation of pigment since occasionally one finds cells in which similar granules and pigment granules are intermixed.

Phagocytosis of haemoglobin by the cells of the guinea pig's omentum is not so pronounced as in rabbit omentum for the reason that the omentum of the former animal contains fewer lymphoid cells[1]). In instances where a number of cells in the small tâches laiteuses of the guinea pig's omentum contained granules, there were also frequently present in the same cell a number of pigment granules. This might indicate a possible relationship between the pigment granules and haemoglobin, although no attempt was made to establish this probable origin.

The free cells of the peritoneal cavity are more phagocytic for foreign haemoglobin than are the fixed cells. This is precisely what one would expect, because the free cells are swimming about in the haemoglobin, and, therefore, they have an opportunity of phagocytosing great quantities of it. Occasionally, however, one finds free mesothelial cells that are very phagocytic. Fig. 14 represents one of these cells: judging from its shape, this cell evidently has been just cut off from the omentum. The variation in the size of the haemoglobin granules is very significant; most of them are large haemoglobin balls. The presence of great numbers of similar balls outside of the cell — not shown in this fig. nor in fig. 12 — indicate that they are phagocytosed as intact balls. Lepehne has also observed the phagocytosis of haemoglobin balls.

On the other hand, the phagocytosis of haemoglobin may result in the formation of large haemoglobin masses which are many times larger than ordinary haemoglobin balls. This is shown in figs. 15 and 16 which represent polymorphonuclear leucocytes. The globular mass shown in the former figure, and the elongated body in the latter, are masses of phagocytosed haemoglobin. Their haemoglobinous origin is particularly evident from the cell of fig. 15. The surrounding halo, which has a pinkish color, represents a mixture of haemoglobin and coagulated lymph.

As in the erythrocytic experiments, nothing was seen which could be interpreted as indicating a relationship between eosinophil and haemoglobin granules. The latter, irrespective of their size and origin, completely disappear. During their gradual digestion they lose their pink

[1]) Weidenreich's studies were based largely on the tâches laiteuses of rabbit omentum. He reports that in one case there was tremendous phagocytic activity on the part of these cells for haemoglobin products.

color (Johnson's stain), become pale, and finally disappear. With Deetjen's method, Giesma stain, it will be recalled, that these granules show quite pronounced changes in their staining reaction during their digestion. With Johnson's stain, however, the granules grow more pale as they approach the final stages of dissolution. A number of these features are shown in the large macrophage of fig. 13. After the disappearance of the granules, the cell-body is studded with vacuoles.

There still remain to be discussed the following questions: 1) do the eosinophil myelocytes of the guinea pig and rabbit omentum contain granules which are of haemoglobinous origin; 2) the source of the mononuclear eosinophils of the peritoneal cavity under normal as well as under experimental conditions.

In the normal, untreated guinea pig and rabbit, as well as in animals that have had repeated intraperitoneal injections of haemoglobin or suspensions of erythrocytes, eosinophil myelocytes are occasionally found in the omentum. These myelocytes frequently contain granules which exhibit basophilic properties. That these granules, however, are not related to haemoglobin is indicated by the presence of other granules in the same myelocyte cell-body that are intermediate in staining reaction. The intermediate tints show that the granules pass through similar developmental stages as those previously described for the granules in the eosinophil leucocytes of rabbit and sheep marrow, and in haemolymph nodes of sheep. Frequently one finds young myelocytes in rabbit and guinea pig omentum in which all the granules are uniformly acidophilic, and no basophilic granules are present. This condition would indicate that the basophilic condition of the granules may be passed through very rapidly. The cell of fig. 17 represents one of these myelocytes; a few of the granules are still small, but all of them possess acidophilic properties. There can be no question about the endogenous origin of these granules.

Granted that Weidenreich, Schott and others are correct in describing numerous mononuclear eosinophils in the peritoneal cavity of the guinea pig, it is necessary to account for their origin apart from those of the blood and marrow. The view expressed here does not maintain that the mononuclear forms of the peritoneal cavity are of a special type. It only attempts to show that if mononuclear eosinophils are present in a given animal, their source is from the omentum, since those of myeloid origin are characterized by polymorphous nuclei. When the eosinophils of the omentum have completed their cytoplasmic differentiations and are thrown into the peritoneal cavity, they retain their rounded nuclei. This seems to be the only logical explanation for the

origin of the mononuclear forms. In the omentum a few intermediate stages between mononuclear and polymorphonuclear forms are found, and the latter are identical with those of the blood-stream.

Dominici describes pronounced local development after the injection of taenia extract into the peritoneal cavity of adult rabbits. This, however, is not the case after repeated injections of suspensions of erythrocytes, haemoglobin, and egg-white. If the omentum were responsible for the tremendous numbers of eosinophil leucocytes following these injections, its activity in the production of these cells would certainly be marked. However, they are not more numerous in the omenta of experimental animals than in normal, untreated ones.

The explanation for the enormous numbers of eosinophil leucocytes in the peritoneal cavity of guinea pigs, after the repeated injections of antigens, is to be found in the response of the organism to anaphylactic conditions (Schlecht 1910 and 1912, Schlecht and Schwenker 1912). In every case where the second injection was made 8—12 days after the sensitizing injection, the animals were anaphylactic. The allergic reaction was so great after haemoglobin injections that the animals usually died five to ten minutes after the second injection. The guinea pig gave the most intense symptoms. According to Hektoen, this animal is "400x as sensitive an anaphylactic reagent as the rabbit".

Every animal that manifested the clinical symptoms of anaphylaxis showed an eososinophilia. Seventeen to twenty-four hours after the second injection, regardless of whether the antigen was a suspension of erythrocytes, haemoglobin, or egg albumen, there was an increase in the number of eosinophil leucocytes of the blood-stream, and a slight increase in the peritoneal cavity. By referring to tables 2 and 3, it is evident from the data that after repeated injections the number of these cells is increased, in the blood and in the peritoneal cavity, over that of the previous experiment. After the fifth injection the eosinophils were numerous in the blood-stream, and extremely abundant in the peritoneal cavity. The explanation for the fact that the blood does not contain enormous numbers of eosinophils is that they are passing out from the circulating blood into the peritoneal cavity with great rapidity.

Sternberg, who repeated Weidenreich's experiments, is, therefore, correct when he states that the increase in the number of eösinophil leucocytes in the peritoneal cavity, following the repeated injections of erythrocytes, is a response to anaphylactic phenomena. He, however, did not attempt to prove that there was an nctual increase in the

number of these cells in the blood, although he did observe that the marrow showed tremendous activity in the production of eosinophils. Stschastnyi noted an increase in the number of these cells in the circulating blood only after the eosinophilia of the peritoneal cavity had subsided. However, if he had made his examinations of the blood from seventeen to twenty-four hours after the injections, he would have obtained different results. Undoubtedly many of the eosinophils of the peritoneal cavity do migrate back into the blood-stream, although others degenerate within the cavity.

From the work of a number of authors it is evident that eosinophilia is closely related to anaphylactic phenomena. Moschcowitz has summarized the observations of numerous authors bearing on diseases of anaphylactic manifestations, and has pointed out that they are all associated with an eosinophilia. Among these diseases he mentions the following: asthma, hay fever, urticaria, exophthalmic goitre, tetany, migraine, epilepsy, and eclampsia. The aetiological relation of helminthiasis to eosinophilia is a well recognized phenomenon.

In order to test Weidenreich's view still further experimentally, injections of haemoglobin were made subcutaneously in the abdominal region of eight different guinea pigs. A number of these animals received only one injection, while others were given two at 8—12 day intervals. Three to twelve days subsequent to the injections, small pieces of subcutaneous tissue were removed as nearly as possible from the region in which the injection was made. These pieces of tissue were spread on cover-slips (Maximow's method), and fixed in Helly's fluid for fifteen minutes. After a thorough washing out of the fixative, the preparations were dehydrated and stained with Dominici's staining mixture.

Following the second injection, pigment granules appeared in fibroblasts, clasmatocytes, large lymphocytes, and 'polyblasts'. Pigment granules and haemoglobin granules were frequently found intermixed in these cells. Since none of the pigment granules were found free, it must be assumed that they represent phagocytosed haemoglobin which has been changed into pigment through the protoplasmic activities of the phagocytes.

Eosinophil leucocytes, in four of the eight experimental animals, made their appearance after the first injection of haemoglobin. There seems, therefore, to be an apparent discrepancy between these results and those obtained after a single intraperitoneal injection of haemoglobin. In this connection it will be recalled that the eosinophil leucocytes were numerous in the peritoneal cavity after the second and subsequent

intraperitoneal injections. A similar increase in the number of these cells took place in the subcutaneous tissue following a second subcutaneous injection of haemoglobin. These eosinophils were identical in their morphological characters with those which appeared in great numbers in the peritoneal cavity after haemoglobin injections.

The eosinophil leucocytes that appeared in the subcutaneous tissue following a single subcutaneous injection, however, were predominantly of the mononuclear type. Three of these cells are shown in figs. 18, 19, and 20. Their general appearance is so strikingly different from that of the eosinophil leucocytes of the blood-stream that one might think that Weidenreich's theory is correct. When these figures, however, are compared with figs. 8, 9, 12, and 14 — and it has been proved that the granules shown in the figures of these cells are not real eosinophil leucocyte granules — it is evident that the granules shown in the cells of figs. 18, 19, and 20 are of a specific type. The preparations from which these cells were drawn contained great numbers of similar cells, and none of them showed anything which would indicate that their granules varied in the least degree from the condition illustrated in the figures.

It has already been proved that phagocytosed haemoglobin granules disappear in the protoplasm of the cells of the peritoneal cavity, and that in the subcutaneous tissue similar phagocytosed granules are transformed into pigment. If the examination of the subcutaneous tissue, however, is made from fifteen minutes to an hour after the injection, a number of cells are found which contain haemoglobin granules only. Never were these granules sufficiently numerous in any cells, however, to account for the great numbers of granules illustrated in the cells of figs. 18, 19, and 20. The fact that cells of this character appeared in preparations which were made twelve days after the first subcutaneous injection show conclusively that their granules are not of haemoglobinous origin. Therefore, the only logical conclusion which we are justified in drawing from studies of the cells just referred to are: these eosinophils are developing in loco: their granules are not of exogenous origin.

Although nothing was observed which might indicate that these granules possessed basophilic or indulinophilic properties at any time, this does not exclude them from representing true eosinophil leucocyte granules. In their staining reaction they are similar to the granules in the fully differentiated eosinophil leucocyte of the blood-stream, and they are also similar to the eosinophil granules in eosinophil myelocytes in rabbit and guinea pig omentum (compare the granules in the

eosinophil illustrated in fig. 18 with similar granules in the myelocyte of fig. 17). It has been proved that eosinophil myelocyte granules are true endogenous formations, and, therefore, it is difficult to understand why the same conclusion should not apply to the granules represented in the eosinophils of figures 18, 19 and 20, especially since there is no evidence for their exogenous origin.

The conditions which are observed in figures 18, 19, and 20, however, are not in accordance with Maximow's claims. He states specifically that the eosinophils of the tissues are identical in all respects with those of the blood-stream. He also claims that there is absolutely no evidence for any genetic relationship between these cells and any of the other cells of the connective tissues. Maximow, therefore, denies the local development of eosinophil leucocytes.

On the other hand, a résumé of the literature on this particular phase of the eosinophil question shows that a number of authors have derived eosinophil leucocytes from various sources. Lymphocytes, large mononuclears, plasma cells, and adventitial cells have all been taken into account as the parent cells of the tissue eosinophils. The present study also shows that these cells are of clasmotocytic origin. In figures 18, and 19, cells are illustrated which favor such an origin. Cells of this character are very common in subcutaneous preparations. If it were not for the eosinophil granules in their cytoplasm, one would not hesitate to diagnose these cells as clasmatocytes. Certainly there can be no question about the clasmatocytic character of the cell of fig. 18. The cells shown in figs. 19 and 20 have been derived from clasmatocytes, because they are the only type of cell in this material which are related to the developing clasmatocyte-eosinophils.

It is evident from the preparations that local development of eosinophils is going on very actively. Despite the fact that Schridde claims that mononuclear eosinophils never occur in the connective tissues, it remains a fact that my preparations show great numbers of mononuclear forms. Although most of these cells are mononuclear, one finds other cells in which the nucleus is slightly intended on one side, and in others it is decidedly bilobed.

Fig. 20 represents a typical mononuclear form. Its nucleus is very similar to that of the elongated cell of fig. 19. In many cases the differentiation of the nucleus does not take place until the cell has reached a rounded form, while in other cases it is distinctly bilobed in cells that are still elongated. Never were the nuclei of the polymorphous type in cells of the type shown in fig. 18. Further investigation will be necessary in order to clear up some of these points. We

are at present, however, justified in concluding that clasmatocytes are capable of differentiating eosinophil granules in their protoplasm. However, there is nothing remarkable about such phenomena in view of what other authors have reported.

Downey finds, in lymph glands of cat, that small and medium-sized lymphocytes, and even lymphocytes which have assumed plasma cell characters, may differentiate into histogenous mast cells. The same author has also proved that histogenous mast cells are differentiating constantly in the connective tissues of adult mammals from non-granular lymphocytes and clasmatocytes. According to Howard and Perkins, large mononuclear leucocytes and plasma cells may differentiate eosinophil granules in their protoplasm.

My preparations showed nothing which could be interpreted as a gradual differentiation of granules. The clasmatocyte-eosinophils were either packed with eosinophil granules or they contained no traces of a similar granulation. In these respects they are similar to the eosinophil myelocytes in the developing thymus of rabbit (Hartmann). In cells similar to the one illustrated in fig. 18, it appears that the granules are all differentiated at one time, because they are of practically the same dimensions. Furthermore, these granules appear to be fully formed elements from the moment of their first appearance in the cell-body; at least nothing was ever seen which might indicate that they possessed basophilic properties.

The present studies of the subcutaneous tissue have shown that clasmatocytes may differentiate into eosinophil leucocytes, although a similar process was not observed in the subcutaneous tissue of six normal, untreated animals. In the experimental animals, however, nothing was seen which might indicate that the eosinophil granules were of haemoglobinous origin. Further studies on the locally developing clasmatocyte-eosinophils will be undertaken in the near future.

IV. General Discussion.

The origin of the eosinophil leucocytes of mammals has been a subject of extensive controversy, the general opinion being that the granules of these cells were of exogenous origin. Studies of the bone marrow of various mammals, however, have proved that the eosinophil leucocyte granules, in the eosinophils of the marrow, undergo a gradual „ripening“ process. The granules of the special cells undergo a similar evolutionary process during their development — a well recognized phenomenon, and interpreted as indicating that the granules are true

protoplasmic differentiations. If this progressive evolution indicates endogenous derivation of these granules, why should not a series of similar changes indicate an analogous origin for the eosinophil leucocyte granules?

In the marrow of normal, untreated animals nothing was seen which might even suggest the origin of eosinophil granules from phagocytosed erythrocyte degeneration products. According to Brass, the marrow may contain haemoglobin, but this does not prove that haemoglobin is utilized in the elaboration of eosinophil leucocyte granules. Granted that these granules were of haemoglobinous origin, it would not necessarily follow that factors which might direct the utilization of free haemoglobin would be operative at all times throughout the entire bone marrow. The possibility, therefore, is not precluded that some parts of the marrow would contain no eosinophils.

The present studies of bone marrow have shown that during heteroplastic regeneration of eosinophil leucocytes the granules of these cells are differentiated gradually in a basophilic protoplasm, and that they undergo changes in their chemical and physical properties before they attain their mature character. These facts prove that the eosinophil leucocyte granules are true endogenous formations, and that they are not fully formed elements when first differentiated in the protoplasm of the cells which contain them. Maximow and Downey, the latter author in particular, have previously emphasized emphatically the true endogenous character of these granules.

In a previous preliminary note (1915a) the writer gave an account of the origin of the mast leucocytes in the bone marrow of the rabbit. In the present study the supposed relationship of mast leucocytes to eosinophil and other leucocytes has been emphasized. In regard to the origin of the mast leucocytes of mammals it is conclusively proved by Maximow, Downey, and the author that these cells form a distinct and independent line of granulocytes which are developed in the bone marrow from non-granular cells. Contrary to the claims of Benacchio, and Pappenheim-Szécsi these cells can always be distinguished from young eosinophils and special cells. Therefore, mast leucocytes are not related to eosinophil and special cells, and they cannot be regarded as „unripe“ eosinophil or special cells, as maintained by several of Pappenheim's students. In their staining reactions, and in their extreme solubility in watery stains, mast leucocyte granules in rabbit marrow are profoundly different in character from eosinophil granules. During their progressive evolution mast granules increase in size, but they have never been known to exhibit changes in their staining pro-

perties at any time. Mononuclear mast leucocytes do not occur in the blood of normal rabbits, and they are not formed from lymphocytes in the circulating blood, as claimed by Pröscher and others.

In regard to the basophilic granules in eosinophil myelocytes Weidenreich admits that a few of these granules may be present in some cells, but he does not interpret them as the precursors of eosinophil granules. It has been shown, however, that the basophilic granules are converted into oxyphilic granules. Not all eosinophil granules, however, are basophilic when first differentiated in the cytoplasm of the myelocyte, but all those that possess basophilic properties are gradually transformed into granules which are oxyphilic. The change in the staining reaction of the basophilic granules may take place while they are still of small dimensions (fig. 2), or it may be retarded and not occur until the granules are of larger size than those in the fully differentiated leucocyte (compare the granules of fig. 2 with those of fig. 4). The subsequent reduction in the dimensions of these larger granules, and the change in the configuration of all the granules corresponds precisely to what Downey describes for similar granules in the eosinophils of guinea pig marrow. All of the facts prove, contrary to the statements of Weidenreich, that the eosinophil granules in the eosinophils of the bone marrow do not appear initially as fully formed elements in the cells that contain them.

The claims of Weidenreich, Badertscher and others, who maintain that the granules of the locally developing eosinophil leucocytes are of exogenous origin are not conclusive. Gütig's claim that there are two different types of eosinophils which are of different origin, the granules of the bone marrow eosinophils representing endogenous formations, while those of the tissues are of erythrocytic origin, is also inconclusive. In this connection it will be recalled that Ascoli failed to connect the local development of eosinophils in haemolymph nodes with fragmentation of erythrocytes, and that Badertscher admits that erythrocyte fragments were never sufficiently numerous to account for all the eosinophil granules in the eosinophils of the thymus. It will also be recalled that Pappenheim and Dominici did not associate the local development of eosinophils with degeneration of erythrocytes, and that Sternberg, who repeated Weidenreich's erythrocyte experiments, could not observe any relation between the pronounced increase in the number of eosinophil leucocytes and destruction of erythrocytes. Hartmann, Maximow, Weill, and Herzog have not attempted to prove or disprove the haemoglobinous derivation of the eosinophil leucocyte granules. It is certain, however, that these

authors, in their studies on the differentiation of the eosinophils in the tissues, would have observed some evidence in favor of the theory if it is true that the granules of these cells are of haemoglobinous origin.

The real difficulty with such a theory is that it is purely a matter of assumption so far as direct evidence in its favor is concerned. The progressive evolution of the granules in the eosinophil myelocytes of the bone marrow, and the series of changes in the granules of the locally developing eosinophils in haemolymph nodes, thymus, and omentum, is also in strict opposition to such a theory. All of these facts support the conclusion, that after these granules have once appeared in the protoplasm of the cells which contain them they undergo a process of "ripening" during which they gradually take on their mature, fully differentiated characters. The life-history of these granules is an exact duplication of what is seen in the bone marrow for similar leucocytes.

Single intraperitoneal injections of haemoglobin gave negative results so far as the eosinophil leucocytes were concerned. An examination of the peritoneal fluid, fifteen to thirty-five minutes after the injection, showed that the macrophages, small lymphocytes, and eosinophils, the latter few in number, contained granules. Heretofore, these granules have been spoken of as haemoglobin granules without proving their haemoglobinous origin. That they are of haemoglobinous derivation is apparent, however, when one recalls that recent work has proved that cells are capable of taking up colloidal suspensions and depositing the particles in the form of granules. Haemoglobin is a colloidal suspension, although it appears in some cases that aggregated particles may be extremely large, as indicated by the presence of large haemoglobin balls in some of the macrophages.

The phagocytosis of free haemoglobin results in the formation and deposition of haemoglobin granules within the cytoplasm of phagocytes. Downey (1915, p. 195), therefore, is correct when he states that . . . . "if the granules of the eosinophil leucocytes . . . . are in any way related to haemoglobin one would be forced to assume that their development is somewhat similar to that of the dye granules in the case of . . . pyrrolblau experiments. The free haemoglobin or its products would be taken up by the protoplasm of lymphoid cells . . . . and after being worked over and combined with the elements of the cytoplasm would be deposited in granular form . . . .".

Downey, and the author, however, have proved that phagocytosis of haemoglobin is not concerned in the formation of eosinophil granules

in the eosinophils of the bone marrow. The author finds that under experimental conditions phagocytosis of free haemoglobin occurs, but this phenomenon is not associated with the formation of eosinophil leucocyte granules. Haemoglobin granules may be very numerous in a cell shortly after the introduction of haemoglobin into the peritoneal cavity, but the granules are transitory bodies. One to two hours after an injection, these granules have completely disappeared from the cytoplasm of the phagocyte.

Phagocytosis of haemoglobin, as well as the phagocytosis of intact red cells, was very pronounced after the repeated intraperitoneal injections of erythrocyte suspensions. The haemolysins caused the foreign red cells to liberate their haemoglobin, whereupon it was phagocytosed and deposited in the cytoplasm of phagocytes in granular form. So far as the ingestion of haemoglobin, with subsequent deposition in the form of granules, is concerned, the results are the same as after haemoglobin injections. The only difference is that phagocytosis of injected haemoglobin is evident after a single haemoglobin injection, while after erythrocyte injections phagocytosis of free haemoglobin does not take place until the animal has developed specific haemolysins for the foreign erythrocytes. Even when haemolysins are present it does not necessarily follow that every red cell gives up its haemoglobin the moment it is introduced into the peritoneal cavity, because many phagocytes may contain a number of red cells that are perfectly normal in shape and staining properties. During subsequent destruction their haemoglobin diffuses into the cytoplasm of the phagocyte: here it is deposited in the form of haemoglobin granules in precisely the same manner as during the ingestion of haemoglobin when introduced intraperitoneally. On the other hand, many phagocytosed red cells do not liberate haemoglobin during their destruction.

Summarizing these facts, it is evident that haemoglobin granules may be formed from injected free haemoglobin; from haemoglobin liberated by haemolised erythrocytes; and from haemoglobin derived from phagocytosed red cells.

All haemoglobin granules are readily distinguished from eosinophil leucocyte granules, and never was anything seen which would indicate that the former gradually transformed into the latter type of granulation. Sooner or later the haemoglobin granules disappear. In only one animal was the writer able to discover these granules in the large macrophages as late as three hours after an intraperitoneal injection of haemoglobin. It is obvious, therefore, that if the examination of the transudate were delayed for a long period of time one might assume

that the increased numbers of eosinophil leucocytes in the cavity were associated with the phagocytosis of haemoglobin products. The fallacy of this assumption, however, is apparent in view of the fact that phagocytosed haemoglobin granules disappear before the eosinophil leucocytes begin to become numerous in the peritoneal cavity.

Weidenreich's conclusions are based on a study of local development of eosinophils in the tâches laiteuses of rabbit omentum. He makes no specific statement regarding the time that elapsed between the last injection of erythrocytes and the examination of the omentum. In all probability the examinations were made shortly after the injections, because his figures indicate that most of the erythrocyte products are predominantly acidophilic. The writer found that phagocytosis was always more marked in the cells of the peritoneal cavity, and this condition probably accounts for the fact that Weidenreich observed pronounced phagocytic phenomena in the omentum of only one animal.

In the omentum many of the phagocytosed haemoglobin granules are undoubtedly changed into pigment, while others are digested. Judging from Weidenreich's figure of cell k (1911, pl. a/b) some of the granules are undergoing dissolution, as is indicated by their staining reaction. Similar conditions were described by the writer in the phagocytes of the peritoneal cavity.

That the increase in the number of eosinophil leucocytes in the peritoneal cavity, following the repeated intraperitoneal injections of antigens, is not the result of local development is conclusively proved by the following facts: 1) the phagocytosis of erythrocyte products by the non-granular lymphoid cells of the omentum does not result in their becoming transformed into eosinophil leucocytes any more than does the phagocytosis of similar products by the macrophages of the peritoneal cavity; 2) the omentum shows nothing which would indicate that it is stimulated in its production of eosinophil myelocytes; 3) that the eosinophils of the peritoneal cavity are of myeloid origin-proved by the eosinophilia of the blood-stream; 5) the eosinophilia of the peritoneal cavity is a response to anaphylactic conditions.

A probable explanation for the great numbers of eosinophil leucocytes in the haemolymph nodes of sheep may be deducted from the results of Fiessinger's recent work on war wounds. He claims that cell autolysis gives origin to proteins which subsequently act as foreign proteins. It therefore seems probable that in haemolymph nodes, where destruction of erythrocytes is supposed to take place, that the split proteins may be responsible for the great numbers of eosinophils.

Fiessinger's claim may also offer an explanation for the local development of eosinophils in the subcutaneous tissue after a single subcutaneous injection of haemoglobin. According to the same author there was a local development of eosinophils in the neighborhood of wounds where split proteins were present.

V. Summary.

The haematogenous mast cells of the rabbit represent a distinct and independent line of granulocytes which is not related to the eosinophil or special leucocytes excepting through the non-granular parent-cell of the bone marrow.

The bone marrow of mammals shows that eosinophil leucocyte granules are real endogenous formations. During their progressive evolution they exhibit changes in their physical and chemical properties. In the early myelocyte stages the granules are small and indulinophilic; later on, however, they increase in size and become oxyphilic. The change in the staining reaction may take place while the granules are still small, or it may not occur until they are of greater size (Downey).

In the marrow nothing was observed which might even suggest that eosinophil leucocyte granules were of haemoglobinous origin. Their complicated life-history shows that they are genuine endogenous differentiations.

Studies on haemolymph nodes and thymus give further support to the view that the eosinophil leucocyte granules are complex structures, and that they undergo distinct changes during their transformation into mature granules. The general life-history of these eosinophils is an exact duplication of what it is in the bone marrow. Nothing was seen in the nodes or thymus which might indicate that their eosinophils contained granules which were related to haemoglobin.

Experimental studies prove that haemoglobin granules are of profoundly different character from eosinophil leucocyte granules. Haemoglobin granules are either digested or changed into pigment, but never are they converted into eosinophil granules.

Repeated intraperitoneal injections of erythrocytes, haemoglobin, or egg-white, at 8—12 day intervals, resulted in an eosinophilia of the peritoneal cavity and blood-stream. The fact that every animal which showed an eosinophilia also manifested the clinical symptoms of anaphylaxis proves that the eosinophilia is a response to anaphylactic conditions.

Single subcutaneous injections of haemoglobin resulted in the differentiation of eosinophil granules in the cytoplasm of clasmatocytes. These granules, however, do not represent phagocytosed haemoglobin products, but are real endogenous formations.

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Explanation of plate.

The figures were outlined with the camera lucida, and they were all drawn with the same magnification: Zeiss homog. immers. objective 2 mm. (N. A. 1.30), and compens. ocular 6.

Figs. 1—6 were drawn from rabbit bone marrow smears. Figs. 1—4 were made from preparations that were fixed in Helly's fluid and stained with Ehrlich's triglycerine staining mixture; figs. 5 and 6 from acetone-lucidol preparations (May-Giemsa stain). Fig. 7 was drawn from a section of sheep haemolymph node; Helly's fixation; triglycerine staining mixture. Figs. 8—16 were taken from peritoneal smears of guinea pig. The following technique was used: figs. 8—11 from smears treated according to Deetjen's method (Giemsa stain); figs. 12—16 from ordinary dry smears; Johnson's stain. Fig. 17 is from rabbit omentum; Helly fixation; Dominici's staining mixture. Figs. 18—20 were drawn from subcutaneous preparations. Fixation and staining the same as in previous figure.

Fig. 1 Myelocyte with indulinophilic granules. A cell of this type is difficult to diagnose, it is either an eosinophil or special myelocyte, probably the former.

Figs. 2 and 3 Eosinophil myelocytes. In fig. 2 notice the great variation in the size of the granules and compare with those of fig. 3. Fig. 2 shows the transformation from indulinophilic to eosinophilic granules. Most of the granules in fig. 3 are eosinophilic, but there are still a few which possess indulinophilic properties.

Fig. 4 Eosinophil leucocyte. All the granules are eosinophilic; compare the shape of these granules with those shown in figs. 2 and 3. In fig. 4 there all still a few rounded granules, but most of them are elongated.

Figs. 5 and 6 Mast myelocyte and eosinophil or special myelocyte which were drawn from the same preparation. Notice the difference in staining reaction of the granules in these two cells.

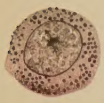
Fig. 7 Eosinophil myelocyte from haemolymph node of sheep; transformation from indulinophilic to acidophilic granules.

Figs. 8—11 show the phagocytosis of erythrocytes and hacmoglobin 45 min. after the third intraperitoneal injection of erythrocytes. In figs. 8, 9 and 11 the large green colored bodies represent freshly phagocytosed erythrocytes; the small greenish granules phagocytosed haemoglobin, figs. 8 and 9. The large faintly lilac colored erythrocyte of fig. 10, and the variously lilac colored haemoglobin granules of figs. 8, 9 and one in fig. 11 show that they are undergoing destruction. Notice that the granules lie in vacuoles in figs. 8 and 9. The extremely small granules near the peripheral portion of the uppermost vacuole in fig. 8 have been formed from an erythrocyte, which subsequent to its phagocytosis, occupied the now vacuolated area.

Figs. 12—16 Phagocytosis of haemoglobin granules, haemoglobin balls, and masses 1 hr. after the second intraperitoneal injection of haemoglobin. Figs. 12 and 13 are large macrophages which contain numerous haemoglobin granules; in the latter fig. the granules are undergoing digestion. Notice the large haemoglobin balls in the free mesothelial cell of fig. 14. Figs. 15 and 16, polymorphonuclear leucocytes containing great masses of phagocytosed haemoglobin.

Fig. 17 Eosinophil myelocyte. This cell was drawn from the omentum of a rabbit which had received repeated intraperitoneal injections of haemoglobin; similar cells were found in the omentum of normal, untreated animals.

Figs. 18—20 Mononuclear eosinophils in the subcutaneous tissue of guinea pig, 12 days after a single subcutaneous injection of haemoglobin. Figs. 18 and 19, clasmatocytic type of eosinophils.



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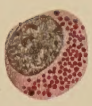

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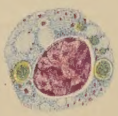

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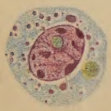

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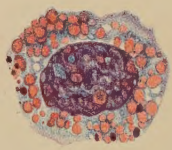

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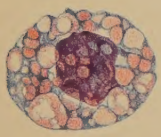

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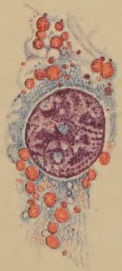

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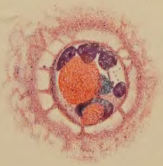

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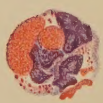

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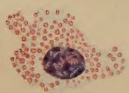

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Ringoen,
Carol E. Young, del.
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LIFE

Adolph R. Ringoen, son of Magnus R. Ringoen* and Martha Maria Ringoen,* was born at Ridgeway, Iowa, January 1, 1887. He graduated from the public schools of Ridgeway in 1904 and entered Luther College the same year. He received the degree of Bachelor of Arts from the University of Iowa in 1909, and the Master of Arts degree from the University of Minnesota in 1913. He was appointed instructor in Animal Biology in the University of Minnesota in 1919.

* Deceased.